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6-Hydroxydopamine and the blood-brain barrier in adult conscious rats

BARBRO B JOHANSSON and MATTS HENNING

Departments of Neurology and Pharmacology University of Göteborg Sweden

JOHANSSON B B & HENNING, M.. 6-hydroxydopamine and the blood-brain barrier in adult conscious rats. Acta Physiol Scand 1980, 110 1–4 Received 3 Sept. 1979 ISSN 0001-6772 Departments of Neurology and Pharmacology University of Göteborg, Sweden

6-hydroxydopamine (6-OHDA), 15 or 50 mg kg^{-1} given as bolus injection to adult conscious rats with aortic catheter rapidly increased mean arterial pressure by 70–78 mmHg. The pressure returned to normal within 40–60 min. The cerebrovascular permeability in rats given 6-OHDA and sacrificed 10 or 60 min later was enhanced as indicated by extravasation of Evans blue albumin and significant increase of ^{125}I human serum albumin content in brain tissue compared to control rats. When the increase in blood pressure was diminished by phentolamine, 6-OHDA treated rats did not differ from controls. It is concluded that the blood pressure elevation induced by i 6-OHDA facilitates the entry of the drug into the brain parenchyma.

Key words Albumin blood-brain barrier hypertension 6-OHDA

6-hydroxydopamine (6-OHDA) is extensively used in experimental physiology and pharmacology because of its selective cytotoxic action on catecholamine-containing neurons. It is generally considered that systemically administered 6-OHDA does not pass the blood-brain barrier (BBB) in adult animals (Laverty Sharman & Vogt 1965 Thoenen 1972 Kostrzewa & Jacobowitz 1974). However it has been reported that the BBB does not completely protect central noradrenergic terminals from the neurotoxic action of large doses (100 mg kg) of 6-OHDA given to adult rats (Sachs 1973 Sachs & Jonsson 1973). An important consideration in this connection is the fact that an injection of 6-OHDA elicit powerful sympathomimetic effects including a rapid rise of the arterial blood pressure (de Champlain & Nadeau 1971 de Champlain & van Ameringen 1972). It is well known that an abrupt increase in blood pressure can lead to a transient dysfunction of the BBB allowing passage of substances including macromolecules into the brain both in conscious and anesthetized animal (Johansson et al 1970 Johansson & Hemming 1976, Johansson 1978). Based on these considerations we have therefore examined the BBB function to macromolecules after v injections of 6-OHDA in adult conscious rats with the aim of elucidating whether the blood pressure increase induced by i.v 6-OHDA can lead to a BBB dysfunction which may allow the drug to pass into the brain also in adult animals.

MATERIAL AND METHODS

Indwelling catheters were inserted under methohexital anesthesia (Brietal Sodium 50 mg kg) into the abdominal aorta via the left femoral artery and in the right jugular vein in 40 male Sprague Dawley rats weighing 200–250 g. The free end of the catheter was exteriorized on the back of the neck. Two days later the aortic catheter was connected to transducer and mean arterial pressure (MAP) recorded in awake, unrestrained rats. Repeated determinations of arterial PCO_2, PO_2 and pH were made. ^{125}I human serum albumin (IHSA, 100 μC kg) and Evans blue (2 ml kg of 2% solution in saline) were given as indicators of the BBB function. Evans blue binds to serum albumin in vivo and is easily detected macroscopically providing convenient gross evaluation of leakage. In addition the staining of the blood provides an indication of the effectiveness of the perfusion (see below). Thereafter 6-OHDA (15 or 50 mg kg freshly dissolved in 0.9% sodium chloride solution containing ascorbic acid 0.2 mg ml) was given as bolus injec

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Table 1 *Mean arterial pressure (MAP) before and after the injection of 6 hydroxydopamine (6-OHDA) or solvent (controls) to conscious unrestrained rats*

$PaCO_2$, PaO_2 and pH before the injection of the drug Mean values ± S E

Experimental group	n	Initial MAP	Maximum MAP	MAP 10 min after injection	MAP 60 min after injection	PaCO (kPa)	PaO (kPa)	pH
10 min								
Control	6	105±3	–	103±4	–	4 6±0 1	11 4±0 1	7.34±01
6-OHDA 15 mg kg	6	98±1	171±1	123±5	–	4.3±0 1	11 7±0	7 33±01
6-OHDA 50 mg kg	6	109±4	187±5	154±4	–	4 4±0	11.8±0..	7 35±0
60 min								
Control	8	105±6	–	115±6	11?±7	4.3±0 1	11 8±0 1	7.35±01
6-OHDA 50 mg kg	6	106±	176±5	160±4	101±5	4.5±0 1	11.5±0 1	7 36±0
Phentolamine 5 mg kg 6-OHDA 50 mg kg	8	96±3	1 4±4	104±4	109±6	4 4±0 1	1 6±0.1	7 36±0

tion Control rats received Evans blue [131]IHSA and the solvent but no 6-OHDA One group of rats was pretreated with 5 mg kg of the alpha-adrenoreceptor antagonist phentolamine 10–15 min before the injection of 6-OHDA to minimize the rise in blood pressure 10 or 60 min after the administration of 6-OHDA the rats were given 30 mg pentobarbital i v and the brains were perfused in situ with saline through the heart for 1 min to remove the tracers from cerebral blood vessel Immediately before the start of the perfusion a blood sample was taken for [131]IHSA determination The brains were divided in telencephalon diencephalon mesencephalon cerebellum and pons+ medulla oblongata (Zeman & Innes 1963) The samples were weighed and the radioactivity determined in a scintillation counter The brain activity was calculated as a percentage of the activity in brain and blood i e 100×[CPM/mg brain tissue over CPM/mg blood]. Statistical differences were evaluated with Wilcoxon rank sum test

RESULTS

MAP before and after 6-OHDA as well as PaCO PaO and pH immediately prior to the blood pressure increase are shown in Table 1 The blood pressure elevation induced by 6-OHDA had a rapid onset and long duration Maximum MAP was reached within 5–25 s and the pressure returned to the initial level within 40–50 min after the high dose (50 mg kg) No change in blood pressure was observed in animals treated with solvent only Phentolamine induced an initial drop in blood pressure of 40–50 mmHg but at the time of 6-OHDA injection the pressure had stabilized on a level 10–15 mmHg lower than initially The pressure response to 6-OHDA was markedly reduced in the

Table 2 *[131]IHSA leakage in the brain after i v injection of 6-hydroxydopamine (6-OHDA) in adult conscious unrestrained rats*

The relative activity is shown as a ratio of activity between brain and blood i e (CPM/mg brain tissue over CPM/mg blood)×100 Mean values ± S E

Experimental group		Telencephalon	Diencephalon	Mesencephalon	Pons Medulla obl	Cerebellum
10 min						
Control	6	0 0..±0 01	0 0 ±0 01	0 0..±0 01	0 0 0 01	0 03_0 01
6-OHDA 15 mg kg	6	0 07±0 01	0 09±0 01	0 08±0 01	0 07±0 01	0 07±0 01*
6-OHDA 50 mg kg	6	0 13±0 03	0 17±0 04	0 13±0 03	0 11±0 03	0 1±0 0**
60 min						
Control	8	0 08±0 01	0 08±0 01	0 09_0 01	0 09±0 01	0 10±0 01
6-OHDA 50 mg kg	6	0 14±0 01	0 19±0 02	0 13±0.0?	0 14±0 01	0 20±0 01**
Phentolamine 5 mg kg 6-OHDA 50 mg kg	8	0 09_0 01	0 11±0 01	0 09±0 01	0 08±0 01	0 09±0.01

* $P<0.05$ ** $P<0.01$ for differences from control rat

group of rats (see Table 1). 14 out of 18 brains from rats given 6-OHDA showed small scattered dots of Evans blue-albumin extravasation. In 7 of these brains—4 belonged to rats given the low dose 6-OHDA—such blue areas were few, i.e. 5 or less. Evans blue-albumin leakage was not observed in controls or in rats pretreated with phentolamine. Except in the latter group, the brains from rats given 6-OHDA had significantly higher radioactivity levels than controls (see Table)

COMMENTS

It is evident from our results that the cerebrovascular permeability to albumin is enhanced after i.v. administration of 6-OHDA in the present doses. The fact that rats pretreated with phentolamine did not differ from controls, indicates that it is the increase in blood pressure rather than any toxic effect of 6-OHDA that is of importance. Moreover, the multifocal appearance of the extravasation is the typical pattern seen in acute hypertension induced by other means (cf. Johansson 1976). The passage of 6-OHDA into the brain is not exclusively prevented by the morphological barrier but probably also by the enzymatic barrier to catecholamines in the endothelial cells, i.e. monoamine oxidase and aromatic aminoacid decarboxylase (Bertler et al. 1966, Hardebo et al. 1979). In the presence of a transient BBB dysfunction it seems that this enzymatic barrier is not sufficient to prevent catecholamines from entering the brain, as indicated by the marked effect on cerebral metabolism induced by noradrenaline and adrenaline after hypertensive or hyperosmotic BBB opening (MacKenzie et al. 1976, Abdul-Rahman et al. 1979) and the presence of amines in the brain parenchyma (Hardebo et al. 1979). There is evidence that adrenaline can pass into the brain at high level of blood pressure to greater extent than would be expected from the macroscopical leakage of Evans blue-albumin (Abdul-Rahman et al. 1979). Hence it is likely that 6-OHDA did enter the brain parenchyma in our experiments. It should be emphasized that transitory BBB dysfunction may occur at relatively moderate blood pressure levels. During normocapnia, MAP levels exceeding 160 mmHg commonly lead to an increased permeability and during hypercapnia protein leakage occurs at even lower pressures (Johansson & Linder 1978). Therefore consideration of arterial pressure is essential in pharmacological and physiological studies using drugs with potential toxic or metabolic effects if allowed access to the brain cells during a transient BBB dysfunction. In this connection it should be recalled that transient increases in arterial pressure may result in asymmetrical BBB dysfunction if one carotid artery is ligated previously, the brain tissue on the ligated side being protected whereas signs of BBB dysfunction may develop on the non-ligated side (Johansson & Henning 1976, Johansson, Linder & Borenstein 1978). Thus, it is preferable to use a catheter in a femoral artery or in the abdominal aorta in this kind of experiments.

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On the sympathetic innervation to the cat's liver and its role for hepatic glucose release

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JÄRHULT J ANDERSSON P-O HOLST J MOGHIMZADEH E. & NOBIN A. On the sympathetic innervation to the cat' liver and its role for hepatic glucose release Acta Physiol Scand 1980, 110 5–11 Received 1 Nov 1979 ISSN 0001-6772. Departments of Physiology Histology and Surgery University of Lund, Sweden and Department of Medical Physiology C, University of Copenhagen, Denmark.

Morphology and function of the adrenergic innervation of the liver were studied in cats. Fluorescence microscopy revealed dense network of adrenergic nerve fibres in association with interlobular vessels and sparse, but unequivocal innervation of the hepatocytes. These parenchymal adrenergic nerve fibres were more frequent in kittens (2 months old) than in adult cats Electrical stimulation of the hepatic sympathetic nerves in the adult adrenalectomized cat evoked small but insignificant increment (1–2 mM) of arterial plasma glucose concentration. When both hepatic and pancreatic sympathetic nerves were stimulated simultaneously arterial plasma glucose concentration increased significantly by about 6 mM We conclude that the pronounced hyperglycemic effect of activation of the sympathetic nervous system in the cat is mediated mainly via an adrenergic influence on the release of insulin and glucgon from the pancreas The sympathetic innervation of the cat liver parenchyma seems to contribute to the hyperglycemia to minor extent only

Key ords Sympatho-adrenal ystem catecholamines, fluorescence microscopy glucose release insulin, glucagon

The mammalian l er is richly supplied with both ympathetic and parasympathetic nerve fibres hich enter the liver mainly via a plexus surrounding the hepatic artery As far as the sympathetic nerve fibres are concerned previous morphological studies ha e reported that these nerves are distributed almost exclusively to the asculature of the iver (Ungvary & Donath 1969 Kyösola 1978) and a number of physiological studies have in great detail described the effects of ympathetic stimulation on he hepatic resistance capacitance and exchange essel (see review by Greenway & Stark 1971) However during the last decade studies on dif erent laboratory animal have suggested that the mpathetic nerves to the liver actually can influ nc also glucose homeostasis in so far that an increased activity in these nerves leads to an incre ment of hepatic glucose release (Shimazu & Amakaw 1968 *b* Edwards 1971 1972 *b* Shimazu & Fujimoto 1971 Edwards & Silver 1972, Järhult 1975 Lautt & Wong 1978 Lautt 1979 Seydoux et al 1979) Recently such an effect of stimulating the hepatic sympathetic nerves has also been shown in man (Nobin et al. 1977 Järhult et al 1979) and refined histochemical techniques have demonstrated a sympathetic innervation of liver cells in monkey and in man (Falck et al 1975 Nobin et al 1978)

This study was undertaken in order to correlate morphology and function of sympathetic nerves in the cat liver Our results indicate that the sympathetic hepatic nerves play a minor role in the control of hepatic glucose release in cats and that activation of the sympathetic innervation promotes glucose output from the liver mainly via adjustments of glucagon and insulin release from the endocrine pancreas.

METHODS

Stimulation experiments 13 cats with an average b wt. of 3.3 kg were sed for the stimulation expts. The animals were fed on standard pellet diet but food was withheld

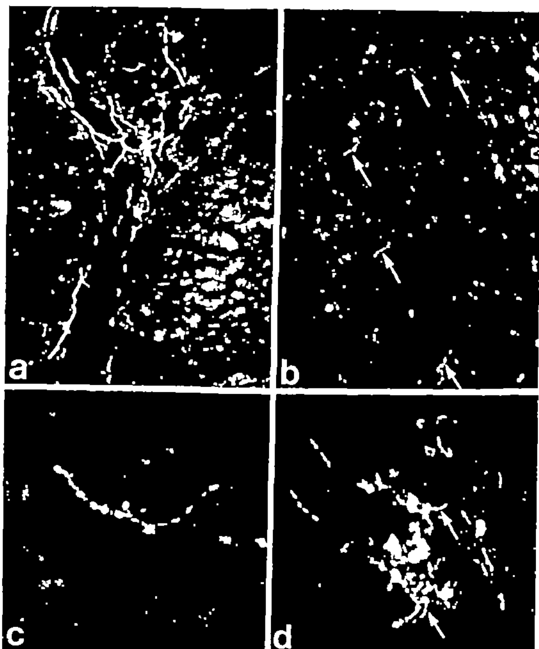


Fig 1 *a–c* are photomicrographs from the liver parenchyma of a two-month-old cat whereas *d* is taken from the endocrine pancreas of an adult cat (*a*) represents the typical noradrenergic innervation to a major artery (×1?0) (*b*) shows the sparse parenchymal innervation Noradrenaline-containing nerve fibres are pointed out with arrows (×1?) (*c*) shows a noradrenaline-containing varicosed nerve fibre running in close contact with the hepatocytes (×180) (*d*) shows noradrenaline-containing nerve fibres in an islet of Langerhans (marked with arrows) Note also the specific fluorescence of the endocrine cells (×180).

for at least 12 h before the expt. Anesthesia was induced with ether and maintained with chloralose (50 mg/kg) and urethane (100 mg/kg) given i A tracheal cannula was inserted The abdomen was opened with a midline incision and the adrenal glands were removed bilaterally The right axillary artery was cannulated and connected to Statham P23AC transducers for recording of mean and pulsatile arterial blood pressure Heart rate was recorded with a tachograph triggered by the systolic pressure wave In 10 cats the hepatic sympathetic nerves were carefully dissected free from the hepatic artery close to its entrance in the liver hilu The nerve fibres were then cut and the distal ends placed in a bipolar platinum ring electrode connected to a Grass stimulator All nerve fibres along the gastroduodenal artery were cut in order to prevent any stimulation of the sympathetic nerves to the pancreas.

The animals were allowed to recover from surgery during 30–45 min The hepatic sympathetic nerves were then stimulated for 10 min with 10 Hz 10 V and 1 ms (6 cats) or for ?0 min with ?0 Hz ?0 V and 1 ms (4 cat ... and

able 1 *Tissue conte t f noradrenaline adrenaline and dopamine in the cat's liver and pancreas*
ata are given as mean values ± S E. and they are expressed in ng/g wet weight 3 in both groups

	Liver			Pancreas		
	Noradrenaline	Adrenaline	Dopamine	Noradrenaline	Adrenaline	Dopamine
month-old cats	457.8±61 5	1.6±0.2	10.6±1.9	254.3±47.5	4 4±1 7	34.6±16 0
dult cats	183 0+24 7	4±1.5	3.5±0 6	251.2±77.0	3±0.5	27.3±13.6

lood samples for determination of plasma glucose concentration ere drawn at intervals before, during and after the stimulation period (see Fig. 2). Atropine (0.5 mg/kg b t) as given 10 min prior to the first control ample Plasma glucose concentration as determined ith the glucose-oxidase method

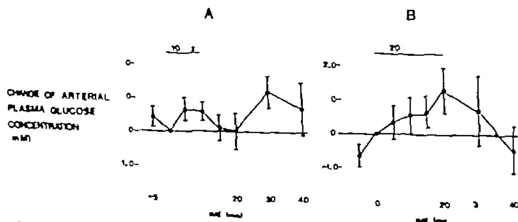
In 3 expts the importance of the pancreatic ympathetic innervation for the glucose response to electrical timulation was demonstrated in the following way Both drenal glands ere removed. All sympathetic nerves were isolated around the celiac artery few mm proximal the bifurcation of the gastroduodenal artery The nerves ere then cut and the distal ends placed in ipolar platinum ring electrode. These nerves consist of fibres destined both for the liver and the pancreas and below they ll be called the hepatic and pancreatic ympathetic nerves After recovery from surgery the nerves were stimulated ith 10 Hz, 10 V and 1 ms for 10 min. Arterial blood samples for determination of serum immunoreactive insulin (IRI) and glucagon (IRG) concentration and of plasma glucose concentration were withdrawn before during and after the timulation. The hepatic ympathetic nerves were then dissected free in the liver hilus as described above cut, and placed in the platinum ring electrode After further resting period of 40 min the same stimulation experiment was repeated. IRI and IRG were determined ith radioimmunoassay as previously described (Ahrén & Holst 1978)

Fluorescence microscopy Liver and pancreas biopsies were taken from 3 anesthetized cats of two months age and from 4 anesthetized adult cats The tissue pieces were frozen in propane-propylene mixture at the temperature of liquid nitrogen. After freeze-drying the preparations were processed for the fluorescence microscopical visualization of biogenic monoamines according to the Falck Hillarp method (Falck et al 1962, Falck 196.., for technical details, see Björklund Falck & Owman 1972). The sections were mounted on microscope slides for fluorescence microscopy

Chemical determinations Noradrenaline dopamine and adrenaline in liver and pancreatic tissue were deter mined by reversed-phase high-performance liquid chromatography using electrochemical detection (Hansson et al. 1979).

Pharmacological treatments T adult cats were pre treated with the MAO-inhibitor nialamid (Niamid Pfizer) One cat was given 10 mg/kg b w t. of nialamid and the other 100 mg/kg b wt. 3 h before it was sacrificed. Liver and pancreatic tissue were then processed for fluorescence microscopy as described above

Four adult cats were treated with 6-hydroxydopamine (6-OH-DA) at dose of 50 mg/kg b wt for 2 consecutive days. After another 6 days the same stimulation expt as described abov was performed. After the end of each xpt tissue specimens were obtained from the liver pancreas and the right auricle of the heart for fluorescence



F 2 Changes of arterial plasma glucose concentration in response to stimulation of the hepatic ympathetic nerves in f adrenalectomized cat given atropine (0 5 mg/kg b wt) Panel A show the results from 9 expts in which the hepatic nerves were activated during 10 min ith 10 Hz, 10 V and 1 ms Panel B shows the results from another 4 expts in which hepatic sympathetic nerves were stimulated during 20 min with 20 Hz 20 V and 1 ms Mean values S E are given

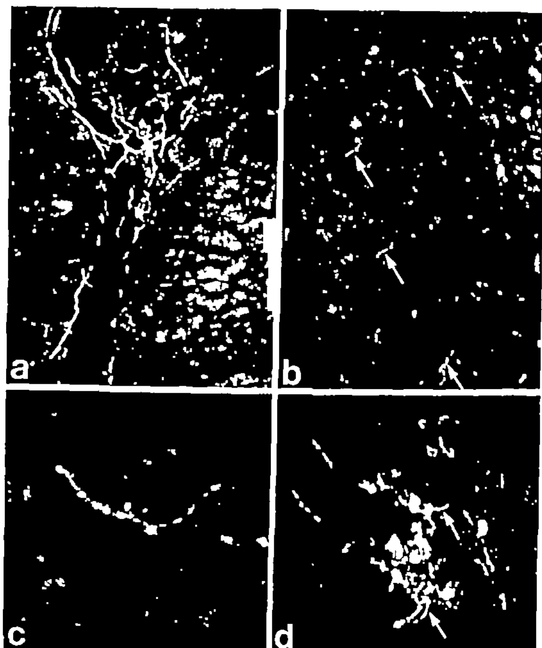


Fig. 1. a–c are photomicrographs from the liver parenchyma of a two-month-old cat, whereas *d* is taken from the endocrine pancreas of an adult cat. (*a*) represents the typical noradrenergic innervation to a major artery (×120). (*b*) shows the sparse parenchymal innervation. Noradrenaline-containing nerve fibres are pointed out with arrows (×120). (*c*) shows a noradrenaline-containing varicosed nerve fibre running in close contact with the hepatocytes (×180). (*d*) shows noradrenaline-containing nerve fibres in an islet of Langerhans (marked with arrows). Note also the specific fluorescence of the endocrine cells (×180).

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oncentration as about 250 ng/g wet weight i e a oncentration similar to that found i the liver of the lult cat Clearcut amounts of dopamine were also etected There was no significant difference be een young and adult cats with regard to the con- ntrations of the amines

Effects of electrical nerv stimulation Fig. 2 ows how the arterial plasma glucose concen- ation was affected by electrical activation of the epatic ympathetic nerves close to the liver hilus ll nervous elements along the gastroduodenal ar ry (destined for the pancreas) were cut in these xpts. Panel A illustrates that arterial plasma lucose concentration increased slightly in response 10 min period of stimulation with 10 Hz 10 V nd 1 ms (peak increment = 0.7±0.3 mM $P<0.1$). anel B show that stimulation of the hepatic nerves ith supramaximal intensity for 20 min (20 Hz, 20 1 ms) caused a peak increment of plasma glucose oncentration by 1.3±0.6 mM ($P<0.1$)

Fig 3 illustrates that different effects on arterial lasma glucose nsulin (IRI) and glucagon (IRG) oncentrations ere observed when the hepatic ympathetic nerves wer stimulated selectively losed circles) or together with the pancreatic erves (open circles) in the adrenalectomized cat. stimulation of the hepatic nd pancreatic sym athetic fibres thus evoked a clear-cut increment of rterial plasma glucose and IRG concentrations as ell as a marked decrement of IRI concentration When the electrode was placed on the hepatic nerves in the liver hilus (distal to the pancreatic nerve branches) lectrical activation with the same haracteristics caused no significant changes in ar terial glucose IRG or IRI concentrations

No changes in ystemic arterial blood pressure or in heart rate was observed in response to electrical stimulation of the hepat sympathetic nerves

Stimulation of the hepatic ympathetic nerves in adrenalectomized cat pretreated with 6-OH-DA did not change the arterial plasma glucose concen- t ation

DISCUSSION

The present fluorescence microscopical study of at liver has shown that the major portion of the mpathetic nerves which enter the liver is dis- tributed to the extra-hepatic blood vessels It was further demonstrated in contrast to previous tudies (Ungvary & Donath 1969 Kyösola 1978) that there is a sparse adrenergic innervation of the liver parenchyma as well. The fact that the latter innervation has not been detected by the previous in estigators might be due to technical difficulties in visualizing tiny adrenergic fibres The existence of a sympathetic innervation to the cat liver paren- chyma is in agreement with our earlier observations in the monkey and in man (Nobin et al. 1977 1978) but the density of the parenchymal innervation in cat liver is clearly lower than that in man and mon- key This quantitative species difference was also reflected in the noradrenaline content of the liver which in the adult cat was 180 ng/g wet weight (Table 1) and in man 970 ng/g wet weight (Nobin et al 1978). Marked species variations in the mor phology of the sympathetic nervation of the liver have also been found in a comparative study on 7 different animals (Moghimzadeh et al., unpub- lished)

Our observation that the endocrine pancreas of the cat recei es a dense sympathetic innervation is in agreement with Cegrell's original report (1968) This morphological finding is further supported by the demonstration of high concentrations of norad- renaline in pancreatic tissue (Table 1) There were also significant amounts of dopamine in the cat s pancreas which most likely emanate from the en- docrine cells known to contain dopamine (Cegrell, Falck & Hellman 1964 Cegrell & Falck 1968 cf Jacoby & Bryce 1978)

In 197? Edwards reported that stimulation of the hepatic sympathetic innervation in the cat caused a prompt increment in the arterial plasma glucose concentration Similarly it was found by one of us (Jarhult 1975) that the hemorrhagic hyperglycemia in cats was markedly depressed by section of the hepatic sympathetic nerves It was further de- monstrated by Edwards that the rate at which glucose was released in response to nerve ac tivation was graded in relation to the stimulation frequency and also depended on the amount of glycogen stored in the liver at the time of the expt. With the same timulation characteristics as used in the present study Edwards found an increment in arterial plasma glucose concentration by 7–10 mM which far exceeds that of 1–2 mM observed in our expts (Fig. 2). This discrepancy between the re- sults might be explained on the assumption that in the tw earlier studies the hepatic *and* pancreatic ympathetic nerves have been stimulated simul- taneously These pancreatic nerve branches emerge

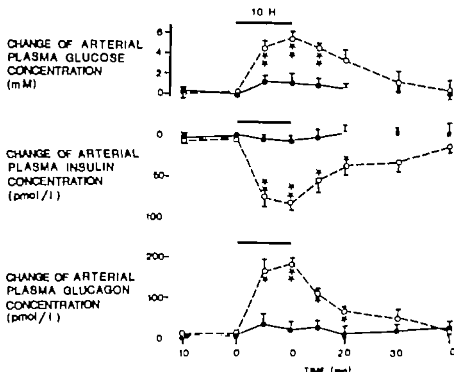


Fig 3 Changes of arterial plasma glucose glucagon and insulin concentrations in response to stimulation (10 Hz 10 V 1 ms) of the hepatic and pancreatic sympathetic nerves (O---O) or to stimulation of the hepatic sympathetic nerves selectively (●—●) The expts were performed on 3 adrenalectomized cats and 60 min elapsed between the end of the first stimulation and the beginning of the second stimulation Atropine (0.5 mg/kg b wt) was given prior to both stimulation periods Mean ± S E are given Asterisks indicate a statistical difference between the two curves $P<0.05$ $P<0.01$

microscopy and chemical determination of noradrenaline adrenaline and dopamine

Statistical methods All data are expressed a mean values ± S E using the Student s *t*-test for the statistical analysis

RESULTS

Fluorescence microscopy Fluorescent varicose nerve fibres were found in the interlobular spaces associated with blood vessels of both portal and hepatic origin (Fig 1*a*) The density of these vessel fibres was comparable in the 2 month-old kittens and in the adult cats In the liver parenchyma scattered varicose sympathetic nerve fibres contacting individual liver cells could be demonstrated (Fig 1*b* *c*) Although the number of fibres were few they were evenly distributed throughout the parenchyma It was observed that the liver parenchyma of the 2-month-old kittens seemed to have a more dense adrenergic innervation than that of the adult cats

After pretreatment with the MAO-inhibitor the intensity of the fluorescent nerve structures increased but no significant increment in the number of nerve fibres was detected

Adrenergic sympathetic fibres were also observed in the islets of pancreas (Fig 1*d*) These nerve fibres were localized in apposition to the endocrine cells and their distribution was similar in the young and adult cats After pretreatment with 6-OH DA the liver and endocrine pancreas were completely devoid of fluorescent nerve fibres Even in the right heart auricle a richly innervated sympathetic tissue all sympathetic catecholamine-containing nerves had disappeared

Chemical determinations The results are summarized in Table 1 The analysis showed that liver tissue from both young and adult cats contained significant amounts of noradrenaline the concentration of which was about twice as high in young as in adult cats After pretreatment with 6-OH-DA the concentration of noradrenaline in the liver fell below the detection level

Table 1 also illustrates the content of the 3 catecholamines in the pancreas The noradrenaline

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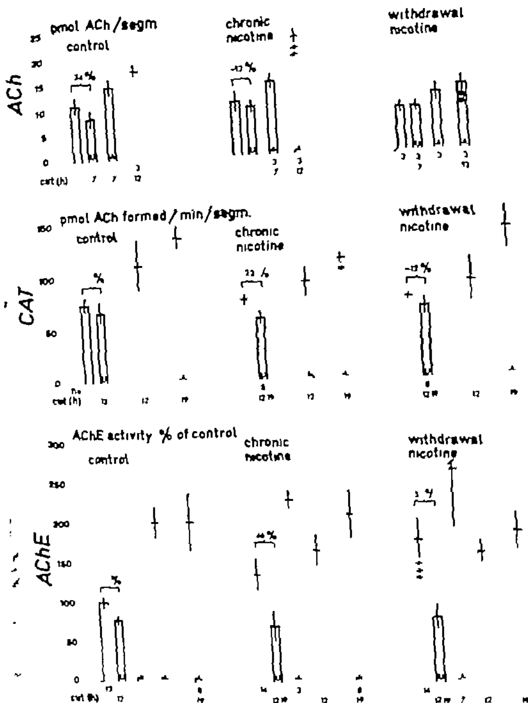
DAHLSTRÖM A BÖÖJ S HEIWALL P-O & LARSSON P A The effect of chronic nicotine and withdrawal on int a-neuronal dynamic of acetylcholine and elated enzymes in preganglionic neuron ystem of the rat Acta Physiol Sc nd 1980 110 13–20 Received 2 Nov 1979 ISSN 0001-6772 Institut of Neurobiology Uni ersi ty of Göteborg Sweden

The effect of chronic nicotine treatment given in the drinking water for 8–10 week in dose equivalent to that of a he y cigarette smoker and of withdrawal 2 days on acetyl-holine (ACh), choline acetyltransferase (CAT) and ACh-esterase (AChE) act ities in the preganglionic cervical nerve and the superior rvical ganglia (SCG) of rats, were tudied Control rat were housed and handled as the nicotine rat After cutting the preganglionic nerve 7 19 h before dissection ACh, CAT and AChE accumulated in the nerv part proximal to the cut (relative to the nerve cell bodies in the spinal cord) A clearance of these substances was observed in the nerve distal to the cut This indicat that all 3 substance are transported proximo-distally in this preganglionic cholinergic nerve In the SCG ACh was decreased already by 7 h (to about 60%), while CAT and AChE-activitie were lowered to 60% and 80 % respectively at 19 h after cutting the nerve Chronic nicotine treatment caused an increased ACh accumulation (by about 35%) and a decreased CAT accumulation (by about 20%) in the cut nerve while the ganglionic level of all 3 substances were essentially unchanged. Withdrawal of nicotine for 2 days prior to the final experiment caused reduced ACh-accumulation (by about 30 %) in the nerve and nor malized the CAT accumulation The AChE-activity of intact nerve was markedly increased to about 175% of control and the transportable fraction of AChE (clearance distal to the cut) was about twice as large as in control In the SCG withdrawal caused marked changes in the ACh content which was decreased to 62 % of control CAT-activity was incre sed to 117% of control while AChE was unchanged Our hypothesis to e plain the r sult s that hronic nicotine treatment and in particular withdrawal of nicotine may cause marked alterations in the act vity of the preganglionic neuron This may induc change in the intra xonal transport of the 3 substances and an increased turnover of ACh in the nerve terminal of the SCG fter withdr wal of nicot ne

Key ord Chronic icotine withdrawal, acetylcholine holinergic enzymes pre ganglionic nerve cervical superior ganglion, intra-axonal transport

Nicotine in small doses has a stimulating effect on autonomic ganglia The drug activates the so-called nicotinic cholinergic receptors on ganglion cells and this effect may be abolished by the ganglionic blocking agent hexamethonium (see discussion in Volle & Koelle 1970) However comparatively low doses of nicot ne may also have a local action on adrenergic and possibly also on cholinergic nerve endings, thus causing a release of the transmitters (cf Trendelenburg 1965 Westfall & Brasted 1972)

Apart from these effects on cholinergic nerve endings and receptors nicotine may also have direct or indirect effects on intracellular events e g on the intra-axonal transport Earlier we have reported effects of acute non-toxic doses of nicotine on acetylcholine esterase (AChE) levels in intact and crushed sciatic nerve of rat In the present paper we have studied the influence of chronic nicotine treatment and withdraw l on the acetylcholine (ACh) AChE and choline acetyltransferase (CAT



3 The effect of chronic nicotine treatment and days withdrawal on acetylcholine (ACh) content (upper part), oline acetyltransferase (CAT) activity (middle part) and acetylcholine esterase (AChE) activity (lower part) in rat rvical sympathetic preganglionic nerve. The values are given per segment intact, A or U (4–5 mm lengths Fig. 2). ChE-values are expressed in % of control intact nerve for each experimental occasion. = number of observations, d cut (h) hour after cutting the nerve. The differences between intact segments and U-segments are indicated in e figure. Vertical bars indicate S.E.M. * = $P<0.05$ ** = $P<0.01$ *** = $P<0.005$ compared to corresponding segments the control group. = $P<0.01$ compared to the nicotine group.

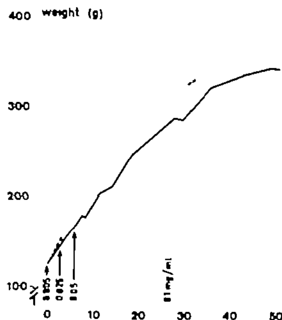


Fig 1 Illustration of increase in body weight of rats during chronic nicotine treatment (——) compared to controls (   ) The arrows indicate changes in nicotine concentrations in the drinking water during a period of 50 days Abscissa days of treatment

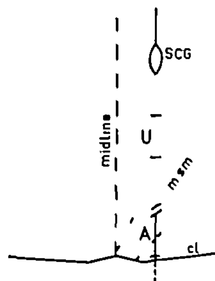


Fig Schematic illustration of the cervical preganglionic nerve and the sympathetic cervical ganglion (SCG) ... The nerve was cut under m sternomastoideus ... The segment A (4–5mm) proximal to the cut (relative to the cell bodies located in the spinal cord) and segment ... (5–10 mm di tal to the cut i e closer to the SCG) ... di sected out 7 1 or 19 hours after operation. In ... ated rats a 17–20 mm length of nerve from 5 mm caudal ... the SCG was dissected and cut into 4–5 mm segments ... analysis. cl=clavicle

levels in the cervical preganglionic neuron system of the rat The dose of nicotine given was chosen to be equivalent to that of a heavy cigarette smoker

MATERIAL AND METHODS

Nicotine treatment Male albino rats (Sprague Dawley) ha ing an initial weight of 130 g and a final weight of about 350 g were used The rats were divided into 3 groups 2 of which received nicotine (Nicotine base Leo) in the drink ing water (tap water) for up to 10 weeks start ng at a concentration of 5 μg/ml reaching 100 μg/ml finally (see Fig 1) The third group (controls) were given plain water All rats were kept 5 in each cage and housed together at ₋5°C on a constant 1 h lighting schedule The water intake (per cage of 5 rat) and the body weight of all groups of animals were recorded at regular ntervals The nicotine solutions prepared from nicotine base were studied regularly by spectrofluorimetric a says of Millipore-filtered samples to control the stability of the nicotine

At the end of the nicotine period each rat drank on the average 27 ml (±4 S D) of a 0 1 mg/ml nicotine solution/24 h, corresponding to an intake of 2 7 mg of nitocine per rat or about 8 mg/kg day Control rats drank about 33 ml/ day/rat One of the nicotine groups was made abstinent by excluding nicotine from their drinking water days before the final experiment (withdrawal group)

Operati e proc ed Under ether anesthesia the cer ical preganglionic nerve bilaterally was cut with a fine pair of scissors just under the m sternomastoideu (Fig. 2). Some rats in each group were used unoperated Seven 1 or 19 h after operation the rats were re-anesthetized and the superior cervical ganglion (SCG) and the pr ganglionic nerve were dissected out The nerves ... placed on an ice-chilled glassplate and cut into 4–5 ... long segments relative to the cut as indicated in Fig ... with the aid of an operation microscope Intact nerve were dissected out from 5 mm caudal to the SCG ... 17–20 mm caudally and cut into 4–5 mm segments ... analysis

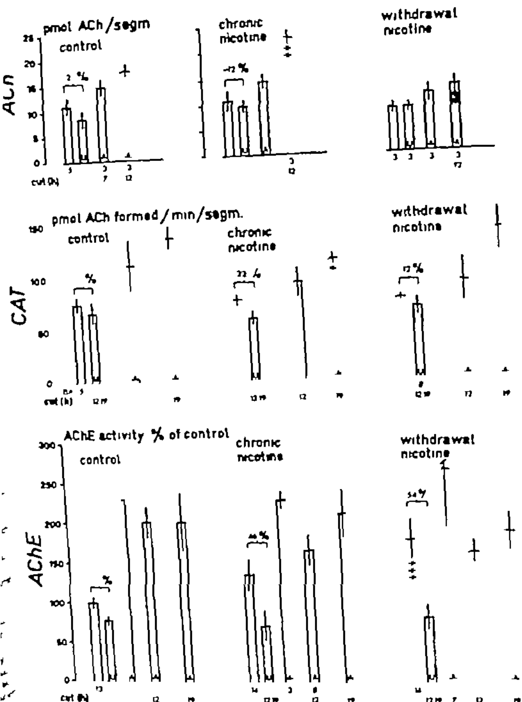
ACh was assayed biologically on a guinea pig ... preparation according to Blaber & Cuthbert (1961). Single ganglia were assayed but 10–30 nerve segments had to be pooled for each ACh assay The activity of the samples ... the gut could be abolished by incubating the samples ... purified AChE (Worthington Biochemical Corporation Freehold N.J) or by treatment of the gut with atropine (10 M) The measured activity thu ACh-like ... will be referred to as ACh n the following

Enzymes were assayed radiochemically using the methods of Fonnum (1969) (AChE) and Glover & Green (197) (CAT) Single ganglia or 6–10 nerve segments (4– mm long) were pooled for each assay

RESULTS

Preganglionic nerve

The *ACh-content* in intact nerve was 11±1 5 pmol per 4–5 mm segment in the control group and 11±1 9 pmol in the nicotine group The withdrawal



3 The effect of chronic nicotine treatment and 2 days withdrawal on acetylcholine (ACh) content (upper part), choline acetyltransferase (CAT) activity (middle part) and acetylcholine esterase (AChE) activity (lower part) in rat cervical sympathetic preganglionic nerve. The values are given per segment intact, A or U (4–5 mm lengths Fig). AChE-values are expressed in % of control intact nerve for each experimental occasion n = number of observations, and cut (h) hours after cutting the nerve The differences between intact segments and U-segments are indicated in the figure Vertical bars indicate S E M = $P<0.05$ = $P<0.01$ = $P<0.005$ compared to corresponding segment the control group ** = $P<0.01$ compared to the nicotine group.

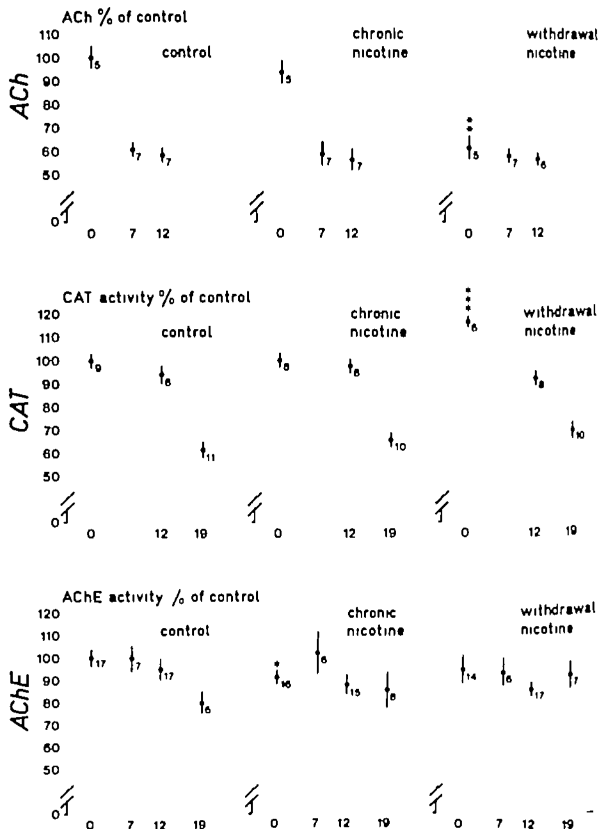


Fig. 4. The effect of chronic nicotine treatment and 2 days withdrawal on acetylcholine (ACh) c nt nt and the activ choline acetyltransferase (CAT) and acetylcholinesterase (AChE) in the rat superior cervical gangl The absc indicated hours after cutting the preganglionic nerve (see F g) The values are expressed in f intact control gang Small figures indicate number of observations. Vertical bars indic te ± S F M. = l 0 05 P 0 005 compared the control group

up had somewhat lower ACh content (8.4±1... ol/segm.). In all 3 groups the ACh content in the segment increased with time after cutting. This rease was higher than control in the nicotine up at 1 h ($P<0.01$) and lower than in the otine group and control after withdrawal 0.01) (Fig. 3). In the U-segment (analysed in an mpt to disclose the "transportable fraction" cf ggendal et al. 1973) small insignificant de ase was observed in the control and nicotine ups, but not in the withdrawal group.

The CAT-activity of intact nerve was about 75 ol of ACh formed/min/4–5 mm segment in all 3 ups. An increase with time in the A segment was served but this increase was at 19 h smaller in nicotine group than control (Fig. 3). The U-ment 5–10 mm distal (i.e. ...) to the cut (see ...) appeared to contain less enzyme activity n the intact segments in each group but the ference was not statistically significant. After onic *nicotine treatment the accumulation of* T at 12 and 19 h was decreased but normalized on after withdrawal.

The *AChE-activity* in intact control nerve varied tween 910 and 4165 pmol of ACh split per min/5 mm segment at the different experimental occasions. The reason for this variance is unknown. erefore, the AChE results are expressed in % of act control nerve at each experimental occasion. each experiment the control value was about 0+7%. In the chronic nicotine group intact rve segments contained 136±20.3% and in the thdrawal group the increase was even larger 8±23.8% ($P<0.005$). The clearance of AChE in e U-segment distal to the cut was 54% after cotine withdrawal as compared to 22% in control d 46% in the nicotine group. The activity of the segment was by 7 h increased t between 230 and 0% of control unchanged but no further increase enzyme activity appeared to occur in this segment between 7 and 19 h (Fig. 3). This is possibly e to accumulation of AChE in more proximal not sayed, segments. No changes were seen after cotine or withdrawal.

...anglia

The *ACh content* (Fig. 4, top) of control ganglia as 273±9.2 pmol of ACh (100±3.7%). After denervation the level was reduced to 61.2% and to 15% at 7 and 12 h respectively. In the chronic cotine group the intact ganglia contained 94% of control ganglia and following denervation the levels decreased to 59% and 56% respectively. Intact ganglia of the withdrawal group had only 6...% of control ($P<0.005$) but the level after denervation were *similar to those in the denervated ganglia of* the two other groups (Fig. 4).

The *CAT-activity* of control ganglia was 79?±...5 pmol of ACh formed/min/ggl (100±3%). At 1 h after denervation the activity was 94% of control and had decreased to 61% at 19 h (Fig. 4 middle). The same pattern was seen in the nicotine group. In the withdrawal group however the level of enzyme activity in the intact ganglia was 117% of normal ($P<0.005$) but following denervation the same decrease occurred as in the other groups.

The *AChE-activity* was estimated on two occasions. 100% on the first occasion was 49541±4095 pmol of ACh split/min/ggl ($n=10$). On the second occasion 100% corresponded to 117109±1019 pmol of ACh split/min/ggl ($n=7$). In the control *group a small decrease was observed at 19 h after* denervation (80%, $P<0.01$). In the nicotine group the AChE-activity of intact ganglia was 97% ($P<0.05$) but no decrease was observed after denervation. In the withdrawal group the ganglia had about the same activity as the control group (Fig. 4 bottom).

DISCUSSION

The ACh content of control preganglionic nerve and ganglia found in the present study as well as the reactions to nerve sectioning correspond well with those of previous study (Häggendal et al. 1973). The CAT-activity of control ganglia (79? pmol 60=47.5 nmol of ACh formed/ggl/h) also compares well with *previous results (e.g. Dibner et* al. 1977: 55 nmol of ACh formed/ggl/h). The AChE-activity of the present study (117 and 49 nmol of ACh split/ggl/min) is of the same magnitude as previously reported (e.g. McLennan 1954: about 84 nmol/ggl/min and Klingman et al. 1968: 28.8 nmol/ggl/min). We have not found values of activity of the cholinergic enzymes in the preganglionic nerve in the literature.

All 3 substances appeared to undergo proximo-distal transport in the preganglionic nerve. This is indicated by the gradual increase in segment A with time after axotomy (Fig. 3) and also by the de*crease* in amount in segment U (distal to the axotomy) 7–19 h after cutting, suggesting a clear

ance due to continued intra axonal transport in the distal nerve stump (cf Ekström & Emmelin 1971) For AChE the increase in segment A was maximal at 7 h This may be due to continued accumulation in segments further proximally (not assayed) or to a reversed transport of AChE in a retrograde direc tion known to occur for AChE in ligated sciatic nerves (e g Bisby & Bulger 1977) The rates of proximo-distal transport of the variou substances cannot be calculated with certainty due to large scatter of the SEM rendering calculation on the transportable fractions of ACh CAT and AChE difficult However the increase in ACh proximal to the cut is of the same order (160%) as in the previ ous study (Häggendal et al 1973 145% above con trol) which may (with a transportable fraction of 5 % Häggendal et al 1973) indicate a transport rate for ACh in the control group of between 112–120 mm/day It is clear that also CAT and AChE under go considerable transport in this nerve this will be further investigated

The effect of axotomy on the ganglia shows that ACh was decreased already 7 h after operation while CAT and AChE activities were significantly decreased at 19 h Since most of the ACh and CAT and a considerable part of the AChE in the SCG is located in nerve terminals of the preganglionic cholinergic neurons (cf Dhar 1958 Gromadzki & Koelle 1965 Joó et al 1971 Tuček 1978) this was an expected finding confirming previous observa tions (e g Banister & Scrase 1950 Eränko & Här konen 1965 Häggendal et al 1973)

The nicotine dose given was chosen to give serum levels similar to that of a heavy smoker According to Wenzel & Azmeh (1970) who also gave nicotine chronically with the drinking water a dose of 4 mg/kg/day is equivalent to that of a heavy smoker In this study we gave about 8 mg/kg/day which is a high dose but not giving any toxic symptoms

The result from the chronic nicotine group dif fered from the control values on 3 point (1) The ACh-accumulation at 12 h was higher than control in preganglionic nerve (2) the CAT activity in the same nerve was lightly reduced at 19 h postopera tively and (3) the AChE-activity in unoperated preganglionic nerve was somewhat elevated (Fig 3) In the ganglia no differences were observed The chronic pharmacological stimulation of the nicotinic receptors in the ganglia during the treatment period thus had no marked effects on the cholinergic sub-

stances in the ganglia The increased 12 h ac[...] lation of ACh in the preganglionic nerve is [...] to the behaviour of the cholinergic enzymes in [...] nerve CAT accumulation was somewhat decrea[sed] (not increased) while AChE activity was incre[ased] (not decreased) Nicotine has been reported to [re]lease ACh from synaptic vesicles (Chiou et [al] 1970) If nicotine had caused a release of ACh [...] from axonal organelles this would have cause[d a] decrease of ACh in the nerve since ACh wa[s] then become available for AChE-degradation [...] an increase as in this study The reason for t[he] increased ACh accumulation is not known at pre[s]ent but may depend on the activity of the neuron

The most dramatic changes occurred after wi[th]drawal of nicotine for 2 days In the nerve the [...] ACh accumulation was much reduced to ne[ar] sub-normal levels the CAT accumulation w[as] normalized and the AChE-activity of intact ner[ve] even more increased to 178% of control va[lue] (Fig 3) In the ganglia ACh content was decrea[sed] to about 62% of control while the CAT-activ[ity] was increased to about 117 of control (Fig 4) These observations may hypothetically be ex[...] plained in the following way After withdrawal [of] nicotine the artificial stimulation of the nicot[inic] receptors cease Due to reflex and local feed ba[ck] mechanisms the release of ACh from the nerve ter[...] minals of the ganglion must increase This can [be] accomplished by an increased impulse activ[ity of] the preganglionic neuron or an increase in t[he] amount of ACh released per nerve impulse or [a] combination of both The decrease in ACh-cont[ent] in the ganglia after withdrawal clearly suggests [a] release of ACh to an extent exceeding the rate [of] ACh synthesi The synthesis on the other hand [is] not likely to be subnormal since CAT-activity wa[s] increased (Fig 4) AChE-activity was unchanged The observations that CAT activity was increa[sed] after withdrawal ugge t that the impulse activity [of] the neuron was raised It i known from other a[u]tonomic cholinergic neuron y tems that the sta[te] of activity of the neuron may determine the activ[ity] of CAT in its nerve terminals (cf Ekström 197[...] Since there is no evidence for the presence of a[c]tivators or inhibitors of CAT (Hemnum 1970 [...] lecki & Brimijoin 1975) the increased enzyme [ac]tivity in rat SCG after nicotine withdrawal sugge[sts] that the supply of enzyme from the soma by mea[ns] of axonal transport i increased The rate of tra[ns]port of CAT in the preganglionic nerve may be [...]

order of 40 mm/d (if the transportable fraction is ut 10%), e 10 times higher than in the motor ves of rat and rabbit (cf Tuček 1975 Saunders al 1973), but only 3 times faster than reported the agus nerve of rabbit (Frizell et al 1970 nnum et al 1973). Since the length of the pre glionic neuron may be about 20–30 mm the reased CAT-activity in the ganglia after days of otine withdrawal may well be caused by an increased somatal synthesis of the enzyme due to creased activity and subsequently increased onal transport to the nerve endings.

The results of the present study show that ronic administration of nicotine in a dose which ates that obtained by hea y smokers causes ochemical changes in an autonomic cholinergic uron These changes may well be secondary to altered functional state of the neuron, caused by nicotine treatment and in particular by withdrawal The clear changes in ganglionic ACh and AT after days of nicotine withdrawal may be ol ed in the well-known abstinence yndrome

pported by grants from the Swedish Tobacco Company os 7310, 7415 7319 7611 7713) and from the Swedish edical Research Council (grants 04X 2207 04P-4173) thank the S edish Leo Pharmaceutical C Hal- gborg (Sweden) for generous supply of nicotine

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ompensatory increase of responses to nerve stimulation the partially denervated rat urinary bladder

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EKSTRÖM J & ELMÉR, M Compensatory increase of responses to nerve stimulation of the partially denervated rat urinary bladder Acta Physiol Scand 1980 110, 1-29 Received 7 Nov 1979 ISSN 0001-6772 Department of Physiology and Biophysics, University of Lund Sweden

The rat urinary bladder was deprived of half of its innervation by removing the pelvic ganglion on one side The motor responses of such partially denervated bladder to stimulation of the pelvic nerve on the other side were examined 1 week, 1 month and months postoperatively On all three occasions the increase in pressure of the operated bladder was larger than that of the control bladder the enlargement was most marked 2 months after operation The responses were further enlarged by eserine and markedly reduced by atropine The present results combined with those of previous investigations showing rapid recovery in the activity of the acetylcholine forming enzyme from reduced level and transient supersensitivity to chemical stimuli after unilateral removal of the pelvic ganglion, suggest that the enlarged responses to nerve stimulation 1 week postoperatively are mainly due to sensitization, while those observed at the later stages are due to collateral sprouting from the cholinergic nerve fibres of the intact pelvic nerve

Key words Collateral sprouting, urinary bladder rat

lorphological and physiological investigations now that the urinary bladder of the rat is well pplied with cholinergic nerve fibres which tra el ot only in the pelvic nerve but also in the hypogasric nerve (Alm & Elmér 1975 Ekstrom & Elmér 977 see Elmér 1978) Most of the fibres of the ypogastric nerve pass through the pelvic ganglion n their ay to the bladder (Langworthy 1965 urinton, Fletcher & Bradley 1973) Bilateral re noval of this ganglion causes the acetylcholinesterase-positive nerves in the bladder to disappear Alm & Elmér 1975) and the activity of the acetyl holine-synthesizing enzyme, choline acetylransferase to fall to 5% or less of the normal value Ekström & Elmér 1977). Unilateral removal of the ganglion is followed by rapid recovery of choline acetyltransferase activity from 58% of the normal activity on the 3rd postoperative day to 86% on the 25th postoperative day (Banns, Ekström & Mann 1978 1979). This recovery in enzyme activity has been suggested to depend on increased motor impulse traffic in the remaining nerves and on out growth of these nerves in the partially denervated bladder (see Ekström 1978) The finding of super sensitivity of the urinary bladder to chemical stimuli 1 week but not 4 weeks after such a partial dener vation seems to be in accordance with these sugges tions (Ekström, Elmér & Banns 1978).

In the present study the electrically evoked motor responses of the urinary bladder on stimulation of the intact pelvic nerve have been examined on dif ferent occasions following removal of the pelvic ganglion on the opposite side Since adrenergic nerve fibres reach the bladder via the same routes as the cholinergic ones (Alm & Elmér 1975) the motor effects of both types of nerves had to be considered However in contrast to the cholinergic innervation, the adrenergic innervation of the detrusor muscle is sparse (Alm & Elmér 1975)

METHODS

A total of 60 adult male rats of a Sprague-Dawley train bred at the Department of Physiology were used Under ethe anaesthesia the abdomen was opened and with the aid

Table 1 *Bladder weights in operated rats 1 week 1 month and 2 months after unilateral removal of pelvic ganglion and in unoperated control rats*

Values are mean ± S E Number of rats is indicated in brackets

	1 week		1 month		months	
	Weight (mg)	Operated/ unoperated %	Weight (mg)	Operated/ unoperated %	Weight (mg)	Operated/ unoperated
Operated	105 6±2 8 (13)	114.3± 6	99 0±3 4 (8)	117 4±5 3	95 8±4 4 (9)	108.7±5
Unoperated	92 9±3 1 (13)		85.2±4.. (8)		89.3±4.5 (9)	

P<0 001 <0 02 >0 1 when the bladder weight of the operated rat is compared with that of the control litter m

of a dissecting microscope the right pelvic ganglion with its nerve filaments close to the bladder was removed The wound was then sutured One week one month or two months after surgery the operated animals and their litter mate controls were anesthetized with chloralose (100 mg/kg) through a femoral venous cannula after induction with ether

Intravesical pressure recording To record the pressure in situ the bladder was exposed and the ureters were ligated A glass cannula was inserted into the bladder through an incision in the urethra and 0 25 ml of physiological saline solution was introduced The pres ure developed by the detrusor muscle was recorded by means of a transducer and a polygraph The resting pressure was about 5 mmHg

Nerve stimulation Using a bipolar electrode the pelvic nerve (including the fibres from the hypogastric nerve which pass through the pelvic ganglion) was stimulated electrically after section just proximal to the pelvic ganglion in some of the control rats the pelvic nerves of both sides were stimulated simultaneously Grass stimulators supplied with stimulus isolation units giving rectangular pulses with a duration of 2 ms either as single shocks or with a frequency of 0 5–30 Hz and 100 Hz and of supramaximal intensity (10V) were used The stimulation period was 15 The stimulation sequence wa repeated after injection of eserine sulphate then after an additional injection of atropine sulphate and eventually after the injection of dihydroergotamine methansulphonate

In the operated bladders the region of the prostatic gland where the ganglion is situated wa inspected no nerve strands were found

At the end of the experiment the bladder wa removed cleaned and weighed

The operated and unoperated litter mates used for comparison were always tested on the same occasion

Statistics Student s *t*-test was used paired comparisons were made between the operated rat and its unoperated litter mate The 0 05 level of probability w s considered signif cant

RESULTS

There was no significant difference in body weights between the operated rats and their controls in any of the three series In the 1 week series the operated rats weighed (mean ± S E) 354±11 g the 13 unoperated rats 354±10 g In the 1-month series the 8 operated and 8 control rats were 361±15 g and 345±12 g respectively and in the month series the 9 operated and 9 control weighed 357±17 g and 368±15 g respectively

In the groups of rats examined 1 week and month postoperatively the partially denervated bladders were 14 and 17 % heavier than their c

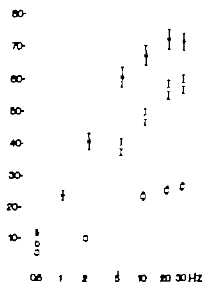


Fig 1 Control bladders The mean pressure increase (and S E) of the urinary bladder of all the 30 control rats in response to various stimul tion frequencies of the pelvic nerve The difference between the response obtained before drug administration and that afte eserine or atropine (in the presence of eserine) wa significant (*P*<0 001) at all frequencies examined The ymbol are as follows ○ before drug administration ✱ after eserine □ after atropine

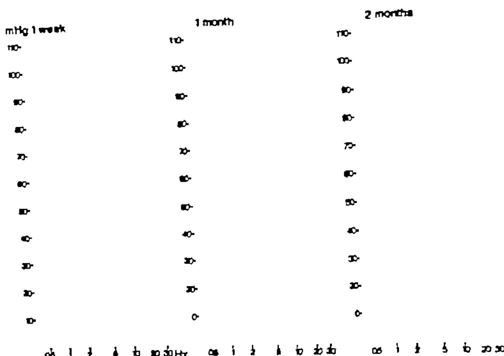


Fig 2 Partially denervated bladders. The mean pressure increase of the urinary bladder in response to stimulation of the intact pelvic nerv 1 week (13 rats), 1 month (8 rats) and months (9 rats) after partial denervation. The difference between the response obtained before drug administration and that after eserine or atropine (in the presenc of eserine) is significant ($P<0.05$ or les), except for the result obtained before and after eserine administration at 20 and 30 Hz months postoperatively. Symbols as in Fig. 1

trol (Table 1). In the -month series the difference of 9% between operated and control bladders was not large enough to be significant

Motor responses of the unoperated bladders. Fig. 1 show the mean (and S.E.) increase in intra esical pressure of the 30 control bladders dev loped in response to electrical stimulation of the pelvic nerve at frequencies arying between 0.5 and 30 Hz before and after administration of eserine and of atropine. In Fig. 3 the pressure rise obtained in response to single electrical shock applied to the nerve is shown (open columns) in this figure the responses are presented separately for each group of observations

The mean increase n pressure in all the controls was 5.9±0.4 mmHg in response to a single shock and 55.3±_8 mmHg in response to 30 Hz: a plateau a eached at 20–30 Hz.

In the presence of *eserine* 0.1 mg/kg, the re sponse to a single shock, 6.1±0.4 mmHg, was not significantly larger than that observed before the administration of the drug however at 0.5–30 Hz the pressure responses after the administration of the cholinesterase inhibitor were larger than before. The responses were particularly enlarged at low frequencies e.g. at 1 and Hz the percentage gain was 85–90 whereas at 20 Hz it was about 30

In the presence of *atropine* 1 mg/kg the effect of eserine on the pressure responses was abolished, and the responses both to ingle shocks and to the frequencies 0.5–30 Hz were found to be s gnificant ly lower than those obtained before the eserine ad ministration they were diminished by 40–55%. The percentage reduction was least at the single shock level where the increase in pressure was 3.6±0._ mmHg.

I order to reveal a possible contribution by the adrenergic nerves to the persisting pressure increase after administration of atropine the stimulation sequence was repeated in the presence of the α-adrenoceptor blocking drug *dihydroergotamine* mg/kg: 100 Hz was used since such a high frequency had previously been found necessary to evoke an α-adrenoceptor mediated contrac

Table I *Bladder weights in operated rats 1 week 1 month and 2 months after unilateral removal of pelvic ganglion and in unoperated control rats*

Values are mean ± S E Number of rats is indicated in brackets

	1 week		1 month		months	
	Weight (mg)	Operated/ unoperated %	Weight (mg)	Operated/ unoperated %	Weight (mg)	Operated/ unoperated
Operated	105 6±2 8 (13)	114.3± 6	99 0±3 4 (8)	117 4±5.3	95.8±4 4 (9)	108.7±3
Unoperated	92 9±3 1 (13)		85.2±4.- (8)		89.3±4 5 (9)	

P<0 001 <0 0_ >0 1 when the bladder weight of the operated rat is compared with that of the control litter mate

of a dissecting microscope the right pelvic ganglion with its nerve filaments close to the bladder was removed The wound was then sutured One week one month or two months after surgery the operated animals and their litter mate controls were anesthetized with chloralose (100 mg/kg) through a femoral venous cannula after induction with ether

Intravesical pressure recording To record the pressure in situ the bladder was exposed and the ureters were ligated A glass cannula was inserted into the bladder through an incision in the urethra and 0 .5 ml of physiological saline solution was introduced The pressure developed by the detrusor muscle was recorded by means of a transducer and a polygraph The resting pressure was about 5 mmHg

Nerve stimulation Using a bipolar electrode the pelvic nerve (including the fibres from the hypogastric nerve which pass through the pelvic ganglion) was stimulated electrically after section just proximal to the pelvic ganglion in some of the control rats the pelvic nerves of both sides were stimulated simultaneously Grass stimulators supplied with stimulus isolation units giving *rectangular pulses with a duration of ms either as single* shocks or with a frequency of 0.5–30 Hz and 100 Hz and of supramaximal intensity (10V) were used The stimulation period was 15 s The stimulation sequence was repeated after injection of eserine sulphate then after an additional injection of atropine sulphate and eventually after the injection of dihydroergotamine methansulphonate

In the operated bladders the region of the prostatic gland where the ganglion is situated was inspected no nerve strands were found

At the end of the experiment the bladder was removed cleaned and weighed

The operated and unoperated litter mates used for comparison were always tested on the same occasion

Statistics Student's *t* test was used paired comparisons were made between the operated rat and its unoperated litter mate The 0 05 level of probability was considered significant

RESULTS

There was no significant difference in body weights between the operated rats and their controls in any of the three series In the 1 week series the 13 operated rats weighed (mean ± S E) 354±11 g and the 13 unoperated rats 354±10 g in the 1 month series the 8 operated and 8 control rats weighed 361±15 g and 345±12 g respectively and in the month series the 9 operated and 9 control rats weighed 357±17 g and 368±15 g respectively

In the groups of rats examined 1 week and month postoperatively the partially denervated bladders were 14 and 17 % heavier than their co

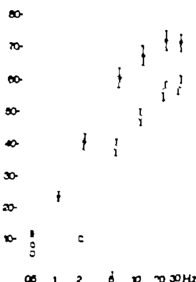


Fig 1 Control bladders The mean pressure increase (and S E) of the urinary bladder of all the 30 control rats in response to various stimulation frequencies of the pelvic nerve The difference between the response obtained before drug administration and that after eserine or atropine (in the presence of eserine) was significant (*P*<0 001) at all frequencies examined The symbols are as follows C before drug administration ■ after eserine □ after atropine

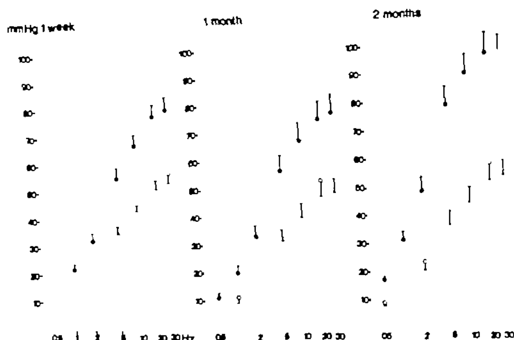


Fig. 4. The mean pressure increase (and S.E.) of the urinary bladder in response to stimulation of the pelvic nerve 1 week (13 rats), 1 month (rats) and months (9 rats) after partial denervation (filled circles) and of control (open circles). The difference between operated and control bladders was significant ($P<0.02$ or less) at all frequencies.

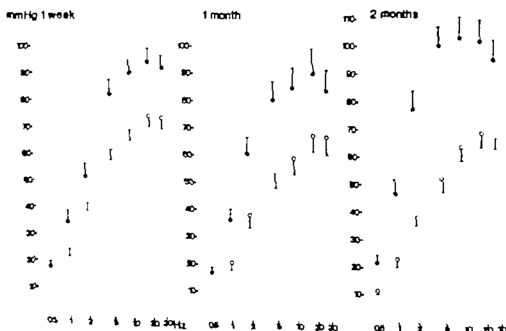


Fig. 5. Eserine. The mean pressure increase (and S.E.) of the urinary bladder in response to stimulation of the pelvic nerve 1 week (13 rats), 1 month (8 rats) and 2 months (9 rats) after partial denervation (filled circles) and of controls (open circles) in the presence of eserine. The difference between operated and control bladders was significant ($P<0.02$ or less) at all frequencies.

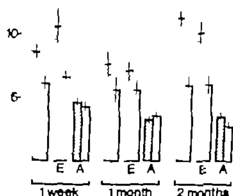


Fig 3 Single shocks The mean pressure increase (and S E) of the urinary bladder in response to single shock stimulation applied to the intact pelvic nerve 1 week (13 rats) 1 month (8 rats) and 2 months (9 rats) after partial denervation (hatched columns) and of corresponding controls (open columns) before and after the administration of eserine (E) and after atropine (A) Comparisons are made between operated and control bladders and when the difference is significant it is indicated as follows $P<0.05$ $P<0.01$ $P<0.001$

tion of the bladder (Elmér 1975*a*) In Fig 8 it can be seen however that at a frequency of 30 Hz the pressure increase was already somewhat lower after the administration of dihydroergotamine than before at 100 Hz the difference was more obvious In the 18 rats examined the pressure increases before and after dihydroergotamine at 30 Hz were 26 1±1.8 and 23 4±1 9 mmHg. At the frequency of 100 Hz the figures before and after the α blocker were 23 7±? 1 and 18 6±2 0 mmHg respectively

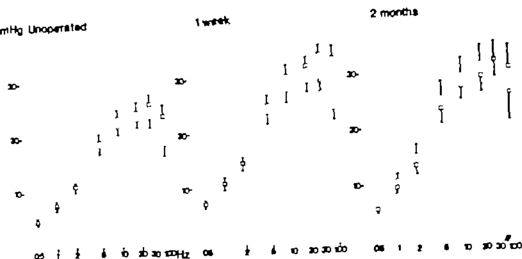
The rat urinary bladder is known to be supplied with β adrenoceptors mediating relaxation (Elmér 1974 1975*a* Ekström 1979) In this type of study however a pressure fall below the resting value was very seldomly found to follow on the pressure rise In those cases where it was observed it did not exceed 1 mmHg

Motor responses of the operated bladders In Fig the mean pressure increases obtained in response to stimulation of the intact pelvic nerve at a frequency of 0 5–30 Hz are summarized at different times after partial denervation before and after eserine and after atropine these curves are also shown in Figs 4–6 where comparisons are made with corresponding controls in these cases S E is indicated In Fig 7 the effect of dihydroergotamine on the pressure of the operated bladders is shown after administration of eserine and atropine The responses to single shock stimulations (hatched columns) are shown in Fig 3

In response to a single shock (Fig 3) the 1-week and 2-month postoperative bladders developed significantly larger pressure rises than their controls while in the 1 month series the enlargement observed only tended to be significant (0 05<P 01) The enlargements expressed as percentages 1 week 1 month and 2 months after operation were 42 37 and 85 respectively In all three series the responses of the operated bladders to 0 5–30 Hz were significantly larger than those of the controls (Fig 4) The largest increases were seen at the low frequencies the smallest at the high In the 1-week series the pressure responses of the operated bladders were enlarged by 72–41 % in the 1-month series by 79–41 % and in the 2 month series by 1?–61 % In the 2 month series the pressure increases found at stimulations of 20 Hz in 4 operated animals were found to be 11? 90 92 and 75 % of those found after combined stimulation of the pelvic nerves on both sides of the 4 corresponding control rats These percentage figures should be compared to those obtained in the control rats when the pressure increase caused by stimulation of the left pelvic nerve is expressed as a percentage of that obtained when both left and right nerves were stimulated simultaneously they were 63 76 68 and 55

In the presence of *eserine* the responses of the operated bladders were also larger than those of the controls both to single shocks and repetitive stimulations At the level of single shocks the response was enlarged by 61 29 and 68 % 1 week, 1 month and 2 months postoperatively (Fig 3) At 0 5–30 Hz the responses were enlarged by 50–5 in the 1 week series by 78–25 % in the 1-month series and by 107–40 % in the 2-month series (Fig 5) The response to single shocks in the operated animals like the controls was not potentiated by eserine neither was the response potentiated at high frequencies in the operated animals of the 2 month series

In the presence of *atropine* the rise in pressure in response to single shocks was about the same in operated and unoperated bladders in the 1 week and 1 month series (Fig. 3) In contrast the operated bladders in the month serie responded with a pressure rise 24 % larger than the controls— a difference which was significant In the 1 week series the responses of the operated bladders were



[Fig]. 8. The mean pressure increase (and S.E.) of the urinary bladder in response to stimulation of the pelvic nerve in the [pr]esence of eserine and atropine (□), and after additional administration of dihydroergotamine (○) in 18 control bladders, [..] bladders partially denervated 1 week in advance and 5 bladders partially denervated [.] months in ad[v]ance. When [sig]nificant the difference before and after dihydroergotamine is indicated as follow [.] $P<0.05$ [.] $P<0.01$ [.] $P<0.001$

[f]ound to be larger than those [i]n the control bladders [w]hen examined at variou[s] times after partial dener[v]ation. The most marked differences in pressure [r]esponses between operated and control bladders [w]ere found in the 2-month series which covered the [lo]ngest time of observation. As judged from the [e]xperiments in which eserine and atropine were [u]sed, the major part of the enlarged responses can [b]e attributed to the activation of cholinergic recep[t]ors. It is well-known in several species including [t]he rat that not all of the rise in pressure developed [in] response to nerve stimulation is atropine sensitive [(]Langley & Anderson 1895, Carpenter & Rubin 1967, Chesher 1970, Carpenter 1977, see also Elmér 1974). A minor portion of the persisting pressure [ri]se at high frequencies of pelvic nerve stimulation [w]as found to be due to activation of α-adrenocep[t]ors in the control bladders at 30 Hz, and in the bladders partially denervated 1 and [.] months in advance at 100 Hz, in contrast to the bladders partially denervated for one week α-adrenoceptors were activated already at 10 Hz.

The outcome of the present expts. combined with that of previous investigations indicates that the intact pelvic nerve gradually extends its field of innervation in the partially denervated bladder. The [e]nlarged motor responses to stimulation of the pel[vic] nerve are maintained, while the initially de[v]eloped sensitization to [.] injection of methacholine and also to noradrenaline disappears (Ekström, Elmér & Banns 1978); the activity of choline acetyltransferase increases (Banns, Ekström & Mann 1978, 1979); this enzyme is confined to the cholinergic nerves of the bladder (Ekström 1975, Ekström & Elmér 1977).

The enlarged motor responses found one week after partial denervation are probably explained mainly by the fact that acetylcholine released on stimulation of the intact pelvic nerve acts on sensitized muscle cells, as seems also to be the case for noradrenaline. It should be mentioned that a selective adrenergic nerve destruction by 6-hydroxydopamine is not followed by an unspecific sensitization towards methacholine (Ekström 1979). By the 6th postoperative day the activity of choline acetyltransferase has increased by 17% from 58% of the activity of the controls found on the 3rd day, an observation which may signal beginning of collateral nerve sprouting and reflect also an increase in the traffic of motor impulses in the intact nerve. One month postoperatively sensitization does not seem to [c]ontribute to the enlarged motor responses, since the bladder at this stage was not found to respond with greater pressure rise than its control after administration of methacholine or noradrenaline; at this time of observation the activity of choline acetyltransferase has increased further, be[i]ng 86% of the activity found in control bladders.



Fig 6 Atropine The mean pressure increase (and S E) of the urinary bladder in response to stimulation of the pelvic nerve 1 week (13 rats) 1 month (8 rats) and 2 months (9 rats) after partial denervation (filled circles) and of controls (open circles) in the presence of atropine (and of eserine) In the series of 1 week and of 2 months the difference between operated and control bladders was significant ($P<0.05$ or less) at all frequencies In the series of 1 month no significance could be demonstrated

significantly larger than those of the controls at 0 5–30 Hz the percentage enlargement being 30–12 % (Fig 6) In the 1 month series there was no difference in the responses between operated and unoperated bladders at these frequencies However in the 2-month series significantly larger responses were again found in the operated bladders compared with those of controls at all frequencies (0.5–30 Hz) the responses being enlarged by 48–29 % and as earlier being most marked at the low frequencies

When comparing the responses of operated bladders in the presence of atropine (and eserine) with those obtained after administration of *dihydroergotamine* (see Fig 8) it was found in the 1 week series that at a frequency of 10 Hz activation of α adrenoceptors already contributed to the response the rise in pressure was 8–14 % less after the α blocker at 10–30 Hz and at 100 Hz it was 27 % less Conversely in the series of 1 and 2 months the responses tended to be larger after the α-blockade than before except at high stimulation frequencies in the 1-month series not shown in Fig 8 response at 30 and 100 Hz was 5 and 1 % less the blockade than before and at 100 Hz in 2-month series 14 % less

In the three series the responses obtained in the presence of dihydroergotamine were significantly larger in the operated than in the control bladders frequencies from 0.5 to 30 Hz with a few exceptions see Fig 7 1 week after operation the responses were enlarged by 44–14 % 1 month after by 45–11 % and 2 months after operation by ?–52 % The mean pressure rise in the operated bladders to single shocks was 18 43 and 79 % of the controls 1 week 1 month and 2 months postoperatively it was only in the 2-month series that the enlargement was significant As in the control bladders a pressure decrease below resting level occurred rarely

DISCUSSION

In the operated bladders the pressure rises evoked by stimulation of the intact pelvic nerve were

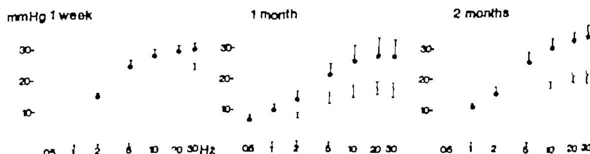
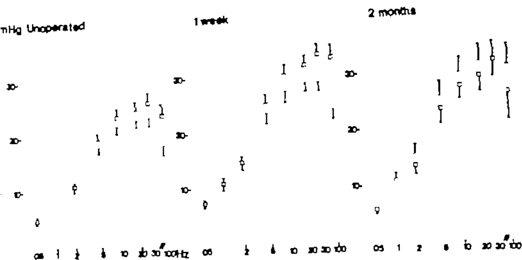


Fig 7 Dihydroergotamine The mean pressure increase (and S E) of the urinary bladder in response to stimulation of the pelvic nerve 1 week (10 rats) 1 month (3 rats) and 2 months (5 rats) after partial denervation (filled circles) and of controls (open circles) in the presence of dihydroergotamine (and of eserine and atropine) The difference between operated and control bladders was significant ($P<0.05$ or less) at all frequencies except at 0 5 Hz in the 1-month series



8 The mean pressure increase (and S.E.) of the urinary bladder in response to stimulation of the pelvic nerve in the presence of eserine and atropine (□), and after additional administration of dihydroergotamine (○) in 18 control bladders, bladders partially denervated 1 week in advance and 5 bladders partially denervated months in advance. When significant the difference before and after dihydroergotamine is indicated as follows $P<0.05$ $P<0.01$ $P<0.001$

und to be larger than those in the control bladders when examined at various times after partial denervation. The most marked differences in pressure responses between operated and control bladders were found in the -month series which covered the longest time of observation. As judged from the experiments in which eserine and atropine were used, the major part of the enlarged responses can be attributed to the activation of cholinergic receptors. It is well-known in several species, including the rat, that not all of the rise in pressure developed in response to nerve stimulation is atropine sensitive (Langley & Anderson 1895, Carpenter & Rubin 1967, Chesher 1970, Carpenter 1977, see also Elmér 1978). A minor portion of the persisting pressure rise at high frequencies of pelvic nerve stimulation was found to be due to activation of α-adrenoceptors in the control bladders at 30 Hz, and in the bladders partially denervated 1 and months in advance at 100 Hz, in contrast to the bladders partially denervated for one week α-adrenoceptors were activated already at 10 Hz.

The outcome of the present expts., combined with that of previous investigations, indicates that the intact pelvic nerve gradually extends its field of innervation in the partially denervated bladder. The enlarged motor responses to stimulation of the pelvic nerve are maintained, while the initially developed sensitization to i. injection of methacholine and also to noradrenaline disappears (Ekström, Elmér & Banns 1978); the activity of choline acetyltransferase increases (Banns, Ekström & Mann 1978, 1979); this enzyme is confined to the cholinergic nerves of the bladder (Ekström 1975, Ekström & Elmér 1977).

The enlarged motor responses found one week after partial denervation are probably explained mainly by the fact that acetylcholine released on stimulation of the intact pelvic nerve acts on sensitized muscle cells as seems also to be the case for noradrenaline. It should be mentioned that a selective adrenergic nerve destruction by 6-hydroxydopamine is not followed by an unspecific sensitization towards methacholine (Ekstrom 1979). By the 6th postoperative day the activity of choline acetyltransferase has increased by 17% from 58% of the activity of the controls found on the 3rd day, an observation which may signal beginning of collateral nerve sprouting and reflect also an increase in the traffic of motor impulses in the intact nerve. One month postoperatively sensitization does not seem to contribute to the enlarged motor responses since the bladder at this stage was not found to respond with greater pressure rise than its control after administration of methacholine or noradrenaline: at this time of observation the activity of choline acetyltransferase has increased further being 86% of the activity found in control bladders.

Two months postoperatively the motor responses were most enlarged e g stimulation of the intact pelvic nerve led to a pressure increase that was about the same as that produced in control bladders by simultaneous stimulation of the two pelvic nerves In normally innervated bladders of the rat a functional overlap of the two pelvic nerves of about 20% has been found at 15–20 Hz (Carpenter & Rubin 1967 Elmér 1975b) Using this percentage figure it can be calculated that the intact pelvic nerve in the operated bladder produced a mean pressure rise that was 5% larger than that obtained in the control bladders if the two nerves had been stimulated at the same time at 20 Hz in all the controls of the 2 month series while in the 1 month series the operated bladder produced a mean pressure rise that was 10% less than that of the controls The activity of choline acetyltransferase did not increase beyond the 1-month stage This may suggest that the sprouting process is complete 1 month postoperatively while during the following period of time changes occurred in the neuro-effector region that increased the efficiency of the transmission of nerve impulses

The present investigation like the previous ones (Banns Ekström & Mann 1978 1979 Ekström Elmér & Banns 1978) shows that in the period that follows operation the bladder gains in weight The gain is lost later in this study no significant difference in the weight between operated and control bladders was found at the latest observation time The bladder weight increase after partial denervation and that after complete denervation (Ekström & Elmér 1977) is probably due to similar causes i e distension of the bladder due to difficulties in bladder-emptying. Thus the disappearance of the slight gain in weight in the series of 2 months may be taken as a further sign of a successful reinnervation

Unfortunately bladders partially denervated by removal of the pelvic ganglion have not been investigated as to changes in the distribution of acetylcholinesterase positive nerves However in such a denervated bladder of the rat an outgrowth of adrenergic nerves from the intact half of the bladder as judged by fluorescent technique has been found to have occurred when examined 6–9 weeks postoperatively (Alm & Elmér 1979). In contrast to the cholinergic the adrenergic nerves do not distribute bilaterally (Carpenter & Rubin 1967 Elmér 1975b) The bilateral distribution of the cholinergic nerves is probably a favourable condition for sprout appears from studies on sympathetic ganglia (M ray & Thompson 1957) and skeletal muscles Edds 1953 Brown & Ironton 1978) organ sively studied that sprouting is favoured when generating and intact nerves intermingle with other In partially denervated skeletal muscle the rat collateral sprouting was demonstrated tologically 4–5 days postoperatively it was particularly abundant during the third week. O connected with the endplates the collaterals w found to mature and early in the second p operative month they resembled normal n fibres A regain in function was found to oc likewise

The motor responses of the rat urinary bla have been reported to be completely atropine-re tant at low frequencies of nerve stimulation, r partially at higher (Elmér 1975 Carpenter 1977) I the present study an atropine sensitive respo was found already at the single shock level both control and operated rats To explain the pa atropine-resistant response it has been suggest that at low frequencies of nerve stimulation acety choline released acts predominantly on recep within the neuromuscular junction not accessible t atropine while at high frequencies of stimula large amounts of acetylcholine also diffuse to rec tors which are sensitive to atropine located furth away from the junction (Chesher 1970). Anoth explanation to the atropine resistancy is the relea of a transmitter other than acetylcholine the pr sence of purinergic nerves releasing ATP has be discussed (Burnstock 1977)

The atropine-resistant responses of the operat bladders were somewhat larger than those of th controls in the 1 week and 2-month series. Se sitization may perhaps be the explanation at th earliest time of observation and at the latest time of observation formation of new neuromuscula junctions less sensitive to atropine

In the operated bladders belonging to the series of 1 and months an α-adrenoceptor mediated in hibitory effect on the atropine resistant respons was found at some frequencies An α-adrenocepto mediated inhibition of the transmission in the parasympathetic ganglia of the cat urinary bladder has been shown by de Groat & Saum (1971) How ever in the rat urinary bladder ganglia are only occasionally found (Carpenter & Rubin 1967 Chesher 1967 El-Badawi & Schenk 1966 Alm &

…er 1975). It may be hypothesized that the out…th of adrenergic nerves in the partially dener…ed bladder has led to a morphological ar…gement favouring an inhibitory influence on the …ve fibres the periphery.

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[illegible] the [illegible] were [illegible] stimulation of the intact pelvic nerve [illegible] a pressure increase that was about the same as that produced in control bladders by simultaneous stimulation of the two pelvic nerves. [illegible] numbers of the [illegible] of the [illegible] has been found [illegible] (Carpenter & Rubin 1967, Elmér 1974). Using this percentage figure it can be calculated that the intact pelvic nerve in the operated bladder produced a mean pressure rise that was [illegible] larger than that obtained in the control bladders if the two nerves had been stimulated at the same time in [illegible] in all the controls of the 2-month series, while in the [illegible]-month series the operated bladder produced a mean pressure rise that was [illegible] less than that of the controls. The activity of choline acetyltransferase did not increase beyond the [illegible]-month stage. This may suggest that the sprouting process is complete [illegible] month postoperatively, while during the following period of time changes occurred in the neuroeffector region that increased the efficiency of the transmission of nerve impulses.

The present investigation, like the previous ones (Bums, Ekström & Mann 1976, 1977, Ekström, Elmér & Burns 1977), shows that in the period that follows operation the bladder gains in weight. The gain is lost later in this study no significant difference in the weight between operated and control bladders was found at the latest observation time. The bladder weight increase after partial denervation and that after complete denervation (Ekström & Elmér 1977) is probably due to similar causes, i.e. distension of the bladder due to difficulties in bladder-emptying. Thus the disappearance of the slight gain in weight in the series of _ months may be taken as a further sign of a successful reinnervation.

Unfortunately, bladders partially denervated by removal of the pelvic ganglion have not been investigated as to changes in the distribution of acetylcholinesterase-positive nerves. However, in such a denervated bladder of the rat an outgrowth of adrenergic nerves from the intact half of the bladder as judged by fluorescent technique has been found to have occurred when examined 6–9 weeks postoperatively (Alm & Elmér 1979). In contrast to the cholinergic the adrenergic nerves do not distribute bilaterally (Carpenter & Rubin 1967, Elmér 1975b). The bilateral distribution of the cholinergic nerves is [illegible] a [illegible] condition [illegible] emerges from studies on sympathetic ganglia [illegible] & Thompson 1957) and skeletal muscle [illegible] 1977, Brown & Ironton 1978), organs [illegible] such studies, that sprouting is favoured [illegible] generating and intact nerves intermingle [illegible] other. In partially denervated skeletal muscle of the rat [illegible] sprouting was demonstrated [illegible] days postoperatively and was [illegible] abundant during the third week [illegible] compared with the [illegible] the collaterals [illegible] formed [illegible] and early in the second postoperative month they resembled normal [illegible] fibres. A return of function was found [illegible] likewise.

The motor responses of the rat urinary bladder have been reported to be completely atropine-resistant at low frequencies of nerve stimulation but partially at higher (Elmér 1975, Carpenter 1977). In the present study an atropine-sensitive response was found already at the single shock level in control and operated rats. To explain the atropine-resistant response it has been suggested that at low frequencies of nerve stimulation acetylcholine released acts predominantly on receptors within the neuromuscular junction not accessible to atropine, while at high frequencies of stimulation large amounts of acetylcholine also diffuse to receptors which are sensitive to atropine located further away from the junction (Chesher 1970). Another explanation to the atropine resistancy is the release of a transmitter other than acetylcholine; the presence of purinergic nerves releasing ATP has been discussed (Burnstock 1972).

The atropine-resistant responses of the operated bladders were somewhat larger than those of the controls in the 1 week and [illegible]-month series [illegible] situation may perhaps be the explanation at the earliest time of observation and at the latest time of observation formation of new neuromuscular junctions less sensitive to atropine.

In the operated bladders belonging to the series of 1 and _ months an α-adrenoceptor mediated inhibitory effect on the atropine-resistant response was found at some frequencies. An α-adrenoceptor mediated inhibition of the transmission in parasympathetic ganglia of the cat urinary bladder has been shown by de Groat & Saum (1971). However, in the rat urinary bladder ganglia are [illegible] occasionally found (Carpenter & Rubin [illegible] Chesher 1967, El-Badawi & Schenk 1966, Al[illegible]

er 1975) It may be hypothesized that the out ...th of adrenergic nerves in the partially dener...d bladder has led to a morphological ar...gement favouring an inhibitory influence on the ... fibres in the periphery

... ork as supported by grants from the Medical ...lty in Lund to J E

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uronal VIP in salivary glands Distribution and release

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UDDMAN R., FAHRENKRUG J MALM L., ALUMETS J HÅKANSON R & SUNDLER F Neuronal VIP in salivary glands Distribution and release Acta Physiol Scand 1980 110:31 38. Received 13 Nov 1979 ISSN 0001-6772. Department of Oto-laryngology University Hospital Malmö the Departments of H tology and Phar macology University of Lund, Sweden and the Departments of Clinical Chemistry Glostrup and Bispebjerg Hospitals, Copenhagen Denmark.

Nerves containing vasoactive intestinal peptide (VIP) were observed in salivary gland of rat, cat and man. VIP nerves were numerous in the cat hile they ere moderate in number in rat and man. The measured concentrations of immunoassayable VIP ere in agreement with the immunohistochemical findings Electrical stimulation of the feline chorda lingual nerve hich stimulates salivary secretion and local blood flow resulted in marked elevation of VIP in the venous effluent from the submandibular gland VIP was not measurable in saliva. Gel permeation chromatography of xtract from cat submandibular gland and from enous plasma collected before and during nervous stimulation revealed one immunoreactive peak with an elution position identical to that of highly purified porcine VIP The finding of neuronal VIP in salivary glands, its release upon nerve stimulation and it known effect on local blood flow support the ew that VIP is neurotransmitter in the salivary gland

Key ord Neuropeptides, vasoactive intestinal peptide (VIP), salivary glands immunohistochemistry radioimmunoassay nerve timulation

soactive ntestinal polypeptide (VIP) is a uropeptide with a wide range of biological ef t Among them are vasodilation (Said & Mutt 70 Larsson et al 1976a Kachelhoffer et al 76) relaxation of mooth muscle (Piper et al. 70 Said et al. 1974) and stimulation of secretion m several exocrine glands (Barbezat & ossman 1971 Said & Mutt 197 Makhlouf & id 1975 Konturek et al. 1976 Domschke et al 77 Lindkaer Jensen et al 1978) VIP containing urones are numerous in the central nervous ys- n (Larsson et al 1976b Fuxe et al 1977 Lorén al 1979) and in several peripheral organs, such as gastrointestinal tract (Bryant et al 1976 rsson et al 1976b Sundler et al 1976 Uddman al 1978) genito-urinary tract (Alm et al 1977 79 Larsson et al 1977 b) and upper respiratory ct (Uddman et al 1978b 1979). The neuronal alization of VIP suggests a role for VIP in urotransmission and evidence is accumulating that VIP is a transmitter (cf Fahrenkrug 1979) In the present investigation we have examined the oc currence and distribution of VIP containing nerves in salivary glands using immunohistochemical techniques and quantified the content by radioimmunoassay Further we have demonstrated a re lease of VIP into local enous effluent upon stimulation of the chorda lingual nerve

MATERIAL AND METHODS

Tissue material

Tissue specimens were taken from the parotid, submaxillary extraorbital lacrimal and sublingual glands of 8 rat and from the parotid submandibular sublingual and zygomatic glands of 14 cats. The rats were killed by decapitation under diethyl ether anesthesia and the cats by sanguination following an overdose of pentobarbital sodium. I addition, tissue material classified as normal by visual inspection was collected from 10 patients under going parotid gland surgery due to benign tumours Salivary gland tissue was also obtained from 10 patients

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undergoing submandibular gland surgery due to ductal obstruction

Immunohistochemical procedures

All specimens were frozen to the temperature of liquid nitrogen in a propane-propylene mixture and freeze-dried. They were then fixed in diethylpyrocarbonate vapour at 55°C for 3 h (Pearse et al 1974) and embedded in paraffin in vacuo. Deparaffinized sections were processed for the immunohistochemical demonstration of VIP using the indirect immunofluorescence procedure (Coons et al 1955) or the peroxidase-antiperoxidase (PAP) technique (Sternberger 1974). The VIP antisera (code no 98P and 5603) were raised against highly purified porcine VIP and have been characterized in detail elsewhere (Said & Faloona 1975, Fahrenkrug & Schaffalitzky de Muckadell 1977, 1978). In cat and man VIP nerves were demonstrated using 5603, while 98P was used in the rat. Antiserum 5603 was used in dilution 1:80 (immunofluorescence) or 1:5280 (PAP staining). Antiserum 98P (a kind gift from Dr S. I. Said, Dallas, Texas, USA) was used in dilution 1:320 (PAP staining). Fluorescein isothiocyanate labelled sheep anti rabbit IgG serum was used in dilution 1:?0 (SBL, Stockholm, Sweden). PAP complex (Cappel Labs, Downington, Pa, USA) was used in dilution 1:3?0. Sections incubated with antiserum inactivated by the addition of excess antigen (30 nmol of highly purified porcine VIP per ml diluted antiserum) served as controls.

Nervous release of VIP

6 adult cats were anesthetized with α-chloralose (80 mg/kg i.v.) after induction with diethylether. Spontaneous breathing was ensured by inserting a tracheal cannula. Rectal temperature was kept at 37–38°C by means of an electrically heated operation table. A glass cannula was inserted into the submandibular duct distal to the point at which it is crossed by the lingual nerve. Chorda lingual nerve was exposed as far centrally as possible and the peripheral end was mounted on bipolar electrodes. Stimuli were delivered by a Grass model S44 stimulator. The nerve was stimulated with square wave pulses with an intensity which was at least twice the threshold value (6–8 V, ? ms). Stimulations in the following order of frequencies: 20, 10, 5, 2.5 and again ?0 Hz, were used with resting period of 15 min between each stimulation. The peripheral end of the cut cervical sympathetic nerve was also stimulated (10 Hz, 4–6 V, ? ms) after the last chorda lingual nerve stimulation. As expected, stimulation of the chorda lingual nerve increased venous blood flow and saliva (Hilton & Lewis 1955).

In order to demonstrate a release of VIP into the local venous effluent during stimulation all tributaries to the external jugular vein, except one or two veins from the submandibular gland, were ligated. Heparin (1 000 I.U./kg i.v.) was given and the external jugular vein was cannulated with a polyethylene catheter. The animal was then left undisturbed for 30–60 min. During each stimulation period 5 ml of blood was collected in ice-chilled tubes containing 2 500 K.I.U. of the protease inhibitor aprotinin (Trasylol, Bayer AG, Leverkusen, FRG). The first 5–6 drops at each stimulation were discarded to avoid contamination from the previous unstimulated period. Between the stimulation periods the venous effluent was returned to the circulation via the femoral vein. The samples were centrifuged immediately after 5 ml of blood was obtained. Plasma was pipetted off and stored at ? until assayed for VIP. Saliva was collected from the stimulated submandibular gland with the same precautions as for plasma.

Radioimmunoassay

The concentration of VIP in salivary gland tissue and plasma was measured radioimmunochemically (Fahrenkrug & Schaffalitzky de Muckadell 1977, 1978). The specimens were immediately frozen on dry ice and weighed. The frozen tissue was stored at -20°C ? extraction of VIP by homogenization at 0°C in a ? homogenizer containing 5 vol. of acidified ethanol (? ethanol containing 0.74% hydrochloric acid ?). ? centrifugation (1 500 g) at 4°C for 15 min the supernatant was decanted and the residue resuspended and homogenized with additional 5 vol. of acidified ethanol. After centrifugation the combined supernatant fractions were evaporated to dryness in vacuo. The samples were reconstituted and diluted in 0.04 M sodium phosphate buffer, pH 7.4, containing 58 µmol human serum albumin (Behringwerke, Marburg am Lahn, FRG) and ? sodium chloride and assayed in 3 different dilutions. Plasma samples were extracted with ? vol. of ? ethanol as previously described (Fahrenkrug & Schaffalitzky de Muckadell 1977).

Gel permeation chromatography

In order to compare the chromatographic behavior of extracted immunoreactive VIP with highly purified porcine VIP (kind gift from Prof. V. Mutt, Karolinska Institutet, Stockholm, Sweden) gel permeation chromatography was performed on extracts of the feline submandibular gland and of plasma from the venous effluent of cat submandibular gland before and during nervous stimulation. The samples were extracted as described above. 500 µl ? mixed with trace amounts of ^{125}I-albumin and ^{22}NaCl ? applied to Sephadex G-50 superfine columns (11×600 mm). Elution was performed at 4°C with 0.25 M ammonium acetate, pH 6.5, containing 77.5 µmol human serum albumin (Behringwerke, Marburg am Lahn, FRG) per liter at a flow rate of 1 ml per h. Fractions of 5 ml were collected and the concentration of immunoreactive VIP in each fraction was measured.

Statistics

Statistical analysis was performed using Student's t-test for paired observation.

RESULTS

Immunohistochemistry

VIP immunoreactive nerves were detected in the salivary glands of all species examined. The distribution of VIP nerves within the gland was markedly patchy with some areas totally devoid of immunoreactive nerves. This was particularly conspicuous in salivary glands of man. The VIP nerve

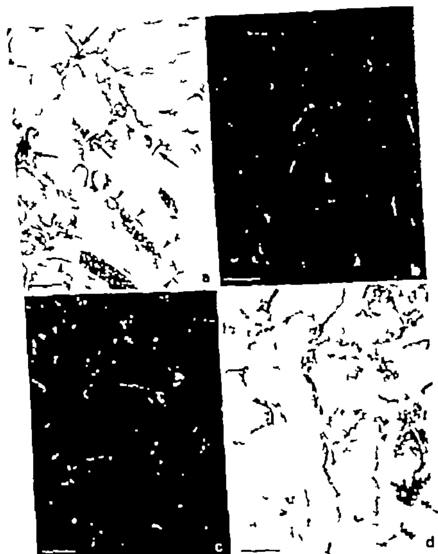


Fig. 1 Distribution of VIP nerves in salivary glands ( ) Rat parotid gland. Few scattered fine varicose VIP nerves in the wall of blood essels (arrow heads) and close to acini (arrows). (*b–d*) Cat sublingual (*b*), submandibular ( ) and parotid (*d*) gland. Rich supply of VIP nerves around acini, blood vessels (–c) and around salivary duct (*d*). ( ) and (*d*) PAP-staining, (*b*) and ( ) immunofluorescence. Marker 50 μm.

were seen to surround glandular acini and to run close to small blood vessels. A notable finding was the accumulation of VIP nerves around salivary ducts where they formed dense plexa beneath the epithelium. Occasionally immunoreactive nerves were observed in the connective tissue troma within the glandular parenchyma. In the *rat* the number of immunoreactive nerves was moderate in all salivary glands (Fig. 1). In the *cat* VIP nerves were numerous in the sublingual (Fig. 1*b*) and submandibular glands (Fig. 1*c*). I the parotid glands (Fig. 1*d*) the VIP nerves had a patchy distribution and were somewhat less numerous they were few in the zygomatic glands. I *man* the frequency of VIP nerves was moderate in both the parotid and submandibular glands.

The main excretory ducts of feline parotid submand bular and sublingual glands received a partic ularly rich supply of VIP nerves (Fig. 2*a*). In the hilus region of the submandibular gland, clus-



Fig 2 Cat submandibular gland (*a*) Main excretory duct surrounded by numerous VIP nerves located beneath epithelium (*b*) Hilar region A small ganglion containing numerous nerve cell bodies and nerve fibres displaying a high degree of VIP immunoreactivity (*a*) Immunofluorescence (×150) (*b*) PAP staining Marker 50 μm

ters of VIP immunoreactive nerve cell bodies were encountered (Fig. 2*b*)

VIP concentration in salivary gland tissue

The concentration of immunoreactive VIP in the various salivary glands of rat cat and man are given in Table 1 On the whole the values agreed well with the histochemical picture The highest concentrations were found in the sublingual and submandibular gland of cats while the parotid and zygomatic glands contained smaller amounts of VIP In man immunoreactive VIP was found in larger amounts in the submandibular gland than in the parotid gland while in rats the concentration was moderate and about the same in the different salivary glands

Nervous release of VIP

Electrical stimulations of the chorda lingual nerve raised the plasma concentration of immunoreactive VIP in the venous effluent (Fig 3) Plasma VIP was unaffected by stimulation of the cervical sympathetic nerve Initial experiments demonstrated VIP like immunoreactivity in saliva This was however later found to be due to protein interference in the radioimmunoassay Consequently VIP does not seem to be released into saliva.

Gel permeation chromatography

Gel-permeation chromatography of extracts from cat submandibular gland (Fig. 4*a*) and of venous plasma (Fig 4*b*) collected before and during chorda lingual nerve stimulation invariably revealed a single immunoreactive peak with an elution position identical to that of highly purified porcine VIP

DISCUSSION

The occurrence of VIP-containing nerves in salivary glands has been noted in previous studies (Bryant et al 1976 Wharton et al 1979) The present investigation confirms and extends these morphological observations In contrast to these a

Table 1 *VIP concentration (pmol/g) in various salivary glands*

Mean ± S E. (*n*) *n* is the number of gland analysed n.d.=not determined –=non-existent

Species	Parotid gland	Submandibular gland	Sublingual gland	Zygomatic gland	Extraorbital lacrimal gland
Rat	11±1 (3)	38±7 (3)	n.d	–	70±7 (3)
Cat	11±3 (15)	223±43 (18)	153±36 (1)	39±6 (1)	–
Man	8±3 (8)	44±3 (8)	n.d	–	–

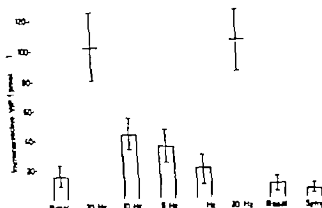


Fig. 3. Plasma VIP in venous effluent from the submandibular gland of 6 cats during stimulation of the chorda lingual nerves or the cervical sympathetic nerve and during unstimulated periods (basal). Vertical bars gives S.E.M. The concentration was increased at stimulation with 20 Hz compared to basal values ($P<0.01$) and also to values obtained with stimulation at lower frequencies ($P<0.05$). Stimulation was performed in the sequence indicated in the figure.

...rs we find great species differences in that VIP ...ves were frequent in the parotid, submandibular ...d sublingual glands of the cat, less frequent in the ...rotid and submandibular glands of man and ...rce in the salivary glands of rat. Species differ...es in distribution have also been noted for adrenergic and cholinergic nerves (Snell 1958, Nor...rg & Olson 1965, Garrett 1966, 1967). On the ...hole the frequency and distribution of VIP nerves agreed fairly well with the concentrations of VIP measured by radioimmunoassay in extract of the salivary glands.

The VIP nerves were distributed around glandular acini and small blood vessels. A particularly rich VIP nerve supply was noted around the intraparenchymal salivary ducts and the main excretory ducts. The presence of VIP immunoreactive nerve cell bodies in ganglia in the hilar region of the sub-

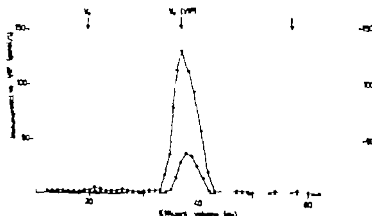


Fig. 4. Elution diagram of VIP immunoreactivity in plasma samples obtained from the venous effluent of the cat submandibular gland before (open circles) and during stimulation (6V, 5Hz, 2 ms) of the chorda lingual nerve (closed circles). Gel-permeation chromatography on Sephadex G-50 superfine column (11 × 600 mm) eluted at 4°C with 0.25 M ammonium acetate, pH 6.5, containing 72.5 μmol bovine serum albumin at a flow rate of 12 ml/h. The column was calibrated with ^{125}I-albumin, ^{22}NaCl and highly purified porcine VIP. V_o, void volume; V_e, elution position of highly purified porcine VIP; V_t, total mobile phase.

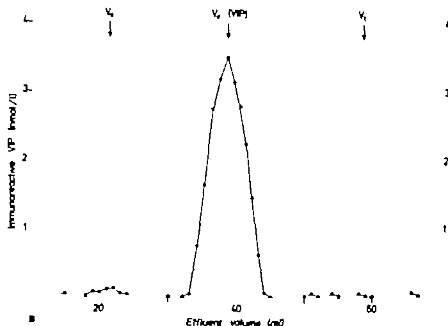


Fig 4b Elution diagram of VIP immunoreactivity from the feline submandibular gland extracted with acidified ethanol (For further details see Methods.)

mandibular gland suggests that at least some of the VIP nerves in this gland may derive from local ganglia Numerous VIP nerves have previously been noted around excretory ducts of other exocrine glands and in particular around sphincters (Alumets et al 1979) It is conceivable that the VIP nerves around the excretory ducts of the salivary glands participate in the regulation of the ejection of saliva and its electrolyte composition (Denniss & Young 1978) Under basal conditions VIP has no stimulatory effect on salivary secretion (Hedemark et al unpublished observations)

Electrophysiological and pharmacological studies have indicated that sympathetic and parasympathetic nerves take part in regulating local blood flow and secretion in salivary glands (cf Burgen & Emmelin 1961) An atropine resistant vasodilation has been observed upon stimulation of the chorda lingual nerve The nervous mediator of this action is unknown (Hilton & Lewis 1955 Bhoola et al 1965 Gautvik 1967 Skinner & Webster 1968) but VIP is a likely candidate based on the following lines of evidence (1) the effect of VIP on salivary blood flow (Shimizu & Taira 1979) mimics chorda lingual nerve stimulation (2) VIP occurs in nerves around the blood vessels of the salivary glands (3) electrical stimulation of fibres in the chorda lingual nerve causes release of VIP in the venous effluent

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anscapillary exchange and distribution of carbon 11 belled ethanol in the isolated perfused rat liver

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KEINÄNEN M., KUIKKA J HESELIUS, S. J SUOLINNA E. M., SOLIN O LÅNGSTRÖM B & NÄNTÖ V Transcapillary exchange and distribution of carbon-11 labelled ethanol in the isolated perfused rat liver Acta Physiol Scand 1980, 110: 39–46. Received 15 November 1979 ISSN 000-000 Medical Cyclotron Project, University of Turku and Åbo Akademi T rku, Department of Clinical Physiology University Central Hospital Kuopio Finland and Department of Organic Chemistry University of Uppsala, S eden.

The d tribution kinetics of ethanol in the isolated perfused rat liver at arious flow rates and ethanol concentrations ere studied by the indicator diffusion technique using ^{11}C labelled ethanol. A double tracer solution with ^{99m}T -labelled red blood cells as the vascular reference and ^{11}C-ethanol as the test substance was injected into the catheter connected to the portal vein Time-activity curves were monitored by NaI(Tl) scintillation detector above the outflow catheter The following values were obtained 0.95±0.01 for the maximal extraction (E_{max}), 3.0±0.1 flow (r=0.98) for the capillary permeability surface area product (PS_{eth}) 18 7±2.5% for the vascular volume (V) and 56.2±9.0% for the ethanol distribution volume (V_e). The volumes were calculated from the products of mean transit time (MTT) and flow (f), and PS_{eth} was calculated from the equation $PS_{eth}=-f\ ln(1-E_{max})$ The esults show that the ethanol distribution in the liver is flow limited since E_{max} was constant at various flow rates (0.67 2.55 ml/g min). The distribution olumes obtained agree well with the values for li er vascular olume and water distribution volume reported in the literature.

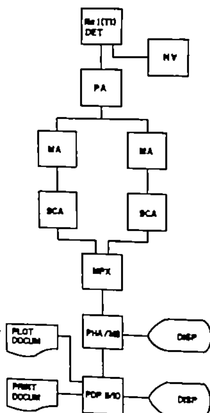
Key ords Ethanol distribution kinetics, liver blood flow capillary permeability diffusible indicators, cyclotron produced isotopes

thanol is a fast diffusible non electrolyte which penetrates cell membranes passively and is distributed in the body to the water distribution volume (e.g. Wallgren & Barry 1970) Absorption dis tribution and elimination of ethanol in the body have been studied by monitoring its blood levels e.g. Widmark 1932 Anthonsen & Crone 1956, Crone 1965) and by tissue analysis techniques e.g. Kulonen & Forsander 1959 Åkesson 1974 Erickson 1976 Scherrer Etienne & Posternak 1963). Since ethanol is eliminated mainly by hepatic metabolism, the study of its metabolism and kinetics in normal and damaged liver is of impor tance There are studies on ethanol consumption in the isolated perfused liver (e.g. Forsander 1960 Gordon 1968) but transcapillary exchange and distribution kinetics have not been investigated

Carbon-11 a short lived cyclotron produced isotope (T 1/2=20 4 min gamma energy 0.511 MeV) has many advantages in biological and medical studies (Tilbury 1974 Wolf 1977) the isotope can be detected externally the radiation dose is small and the experiment can be repeated after short intervals. Synthesis of C-labelled ethanol is relatively simple but thus far its experimental use has been scarce De Grazia et al (1975) have given brief report on the whole body distribution of C ethanol in cats. Raichle et al (1976) have statet that C-ethanol extraction during the first capillary passage was 97% in the brain of monkeys Robinson et al. (1973) have tudied ^{11}C-ethanol extraction in the brain and Poe et al. (1973) in the heart, but they do not introduce quantitative results.

We use ^{11}C-ethanol to investigate extraction and

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3 Flow chart of the radioactivity counting device and computer hardware Abbreviations: NaI DET = detector HV high voltage supply PA = preamplifier MA = main amplifier SCA single channel analyzer MPX = multiplexing unit, PHA/MS = pulse height analyzer in multiscaling mode PLOT = plotter DISP display PRINT line printer PDP 11/10 computer

amplifier and two single channel analyzers using multiplexing unit (Fig 3) The whole photopeaks were used in window setting A 4096-channel pulse height analyzer multiscaling mode (Nuclear Data Inc) was used for recording. A channel width of 0 4 was used The whole measuring time was 5 min. From the memory of the pulse height analyzer the data were transferred to PDP 11/10 computer (Digital Equipment Corp Ltd) and stored on magnetic disc For the calculations corrections were made the background counting rates, physical half-life of rbon-11 and overlapping of ^{14}C-activity in the T -window (9%) The catheter delay was corrected according to Bassingthwaighte (1966) The peak counting rates were about 4 000 counts/0 4 for ^{99m}T -red blood cells and 2 000 counts/0 4 for ^{14}C-ethanol.

Calculations

The radioactivity-time curves for ^{99m}T -red blood cells and ^{14}C-ethanol are normalized to unity area to give the transport functions $h_R(t)$ and $h_D(t)$ used in the calculations. The digital data were analysed with a Basic program. The following parameters were calculated the maximal ethanol extraction (E_{max}) and capillary permeability surface area product (PS_{max}) (Crone 1963) mean transit times (MTT) and mean transit time distribution volumes (V = ^{99m}Tc-RBC distribution volume V = ^{14}C ethanol distribution olume) (Goresky 1963 1970 and Bassingthwaighte 1970 1974). The following equations were used:

(1) Instantaneous extraction,

$$(E(t)) \quad \frac{h_R(t) - h_D(t)}{h_R(t)}$$

where $h_R(t)$ = the transport function of the reference substance (the non-diffusible tracer) (s), $h_D(t)$ = the transport function of the test substance (the diffusible tracer) ( ).

(2) Capillary permeability surface area product

(PS_{max}) = −f ln(1−E_{max}), where f = liver perfusion flow (ml/g min).

(3) Mean transit time

$$(MTT) = \frac{\int^{\infty} th(t)\,dt}{\int_0^{\infty} h(t)\,dt}$$

where t = time (s), h(t) = the transport function ( )

(4) Distribution volume of the tracer concerned (V) = f MTT (% of the total volume of the liver tissue) The total volume of the liver tissue was calculated from the weight of liver (Goresky 1963)

(5) Distribution coefficient

$$(\gamma) = \frac{V - V}{V}$$

where V = distribution volume of test substance, V = distribution volume of reference substance

RESULTS

Responses to 3? injections of the double tracer solution containing ^{14}C-ethanol and ^{99m}Tc-labelled red cells were obtained in 7 isolated rat livers During one perfusion expt. 3–5 injections were carried out. The duration of the perfusion expt. varied from 30 to 45 min depending on the number of ethanol injections The oxygen consumption of the perfused liver was constant for at least 90 min from the beginning of the expt. The mean consumption was 05±0.26 µmol/g × min^{-1} (N = 5 4 samples per rat were taken 20–90 min after the perfusion was begun). Two typical runs are shown in Fig. 4 following the single bolus injection the transport functions $h_R(t)$ and $h_D(t)$ were recorded over the outflow catheter and the instantaneous extraction (E(t)) calculated from equation (1) The appearance and mean transit times of reference and test substances were much longer at low flow rates. The

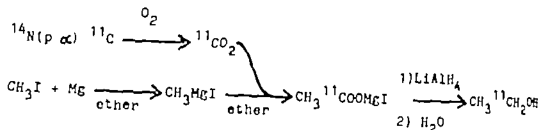


Fig 1 Scheme of the synthesis of 1-[C]-ethanol

distribution kinetics of ethanol in the isolated per fused liver at various flow rates and concentrations by the indicator diffusion technique (Chinard et al 1955 Crone 1963)

MATERIALS AND METHODS

The synthesis of 1-[¹C]-ethanol

C was produced by the ¹N(p α)¹ C reaction using a gas target containing N_2 with less than 0.5% O_2. The irradiations were carried out at the 103 cm cyclotron of Åbo Akademi Turku (18 MeV protons external beam of 50 µA current) In a typical run ¹CO was obtained in a yield of 200 mCi/0.5 h with a 1 MeV beam of protons of 10 µA current.

The synthesis of 1-[C]-ethanol was performed by a procedure earlier used (Raichle et al 1976) slightly modified according to Fig 1 The produced ¹CO was trapped in 1 5 ml of a 0 5 mol/l methyl magnesium iodide solution in ether (the Grignard solution was freshly prepared) After the trapping the solvent was evaporated and the residue was reduced with 3 ml of lithium aluminum hydride in ether (7 mg/ml) After another evaporation the residue was hydrolysed with water The ethanol was trapped on transfer in a glass tal cooled with liquid N

The radiochemical and chemical purity was analysed by radio-gas chromatography The analys s was performed with a 1 5 m Porapak Q column (80–100 mesh) on an Aerograph 600-C gas chromatograph (Wilkens Instr Res Inc) The radioactivity was detected with a NaI(Tl) scintillation detector Nitrogen wa used as carrier gas with a flow rate of 70 ml/min The analysi was carried out at a temperature of 135°C

More than 90% of the analysed activity was found in the ethanol peak the rest found in the methanol peak The time used for the synthesis starting from CO_2 was 20 min including analysis The total yield of 1-[¹C]-ethanol was 50% corrected for decay The use of carefully dried equipment throughout the ynthesis was essential to avoid formation of impurities

Perfusion experim nts

Male W star rats (age 3–4 months) were used in the per fusion experiments The mean weight wa 400 g (range 360–430 g) The open perfusion system designed according to Hems et al (1966) is shown in Fig. The perfusion pressure was regulated by changing liquid level The Krebs bicarbonate perfusion buffer (Alabaster & Bakhle 1970) was continuously bubbled with carbogen ga (O_2 and 5% CO) The rat was given 0 2 ml heparin (IU/ml) and anesthetized with ether The abdomen opened and cannulas inserted into the portal vein and cava inferior The vena portae cannula was then c nected to the perfusion system and the vena cava c to the outflow The liver was perfused for 10–20 r before injections The perfusion pressure and flow were measured The tracer mixture (0.2 ml) conta about 50 µCi C-ethanol and about 100 µCi ⁹⁹ᵐT blood cells (⁹⁹ᵐTc RBC) in physiological saline prep according to Bardy et al (1975) A bolus injection given into the inflow tubing close to the liver The w content of liver tissue was calculated from the differ of wet and dry weight

The viability and function of the perfused liv tested by determinations of pO_2 pCO_2 and pH of p fusion fluid from inflow and outflow tubings

Measurement of radioactivity

The radioactivities of ⁹⁹ᵐTc-labelled red blood cells C-ethanol were monitored over the outflow catheter NaI(Tl) scintillation detector at a distance of 35 cm f the liver The signal from the detector was taken throu

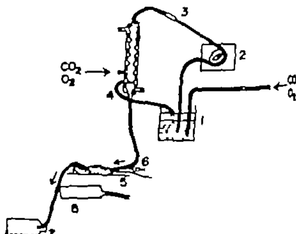


Fig 2 Schematic diagram of the perfusion system (1) Buffer reservoi (at 37°C bubbled with carbogen) (2) roller pump (3) filter (4) oxygenator (at 37°C also bubbled with carbogen) It is an open ystem where the height of the liquid level determines the perfusion pressure (5) platform for rat (6) injection site (7) bottle for collecting outflow (8) NaI(Tl) detector

1 *Li e ight extraction and d strib tion olumes of* ^{99m}*Tc-red cells and C-ethanol i perfusion ment*

reviations are explained in the text

ment	Liver eight (g)	E_{max}	V (%)	V (%)	7
riments ithout addition non radioactiv ethanol[a]	13 4±0 3	0 95±0 01	18 7±..5	56 9.0	09±0 75
riments ith addition non radioactive ethanol[b]	13 4 0.3	0 95_0 01	19..±..6	57....9	04±0.54

perfusion xpts on 7 animals.
erfusion expts on 3 animals

t of flow and concentrat on on ethanol action nd apill ry permeabil ty— face area product

experiments the flow of perfusion fluid ranged m 0 67 ml/g × min to ..55 ml/g × min In this ge E_{max} remained constant (0 95±0 01) indicat that flow does not influence the maximal ethanol raction (Table 1) The shortening of the plateau se of the instantaneous extraction curve (Fig.) can be explained by faster back diffusion of anol from liver tissue to intravascular space with gher flow rate

In some experiments non radioactive ethanol was ded to the perfusion fluid to a final concentration 0 38±0 07% This increase in the concentration ethanol did not change the maximal extraction lue (Table 1). The radioactivity of the liver was llowed by means of a separate gamma-ray onitor externally in 1 expts to determine the nount of tracer which remained in the liver The ean fraction of C-radioactivity remaining in the er after single passage was 6% (range 2-8%) of e injected dose At high flow rates (>2 ml/g × in) the fraction of C-radioactivity remaining in e liver w s smaller (?%) than at low rates (<1 l/g min^{-1} 8%). This fraction is not detected, hen radioactivity is measured above the outflow bing In calculations it should be taken into ac ount

The relation between the flow rate and capillary ermeability surface area product is shown in Fig. Value of PS_{eth} increases linearly as a function of low rate (f). The equation describing the regression ne is $PS_{eth}=3\ 0\pm0.1\times f$ (r = 0.98) The calculated PS_{eth} values ranged from 1 76 ml/g × min at low low rates (f = 0.67 ml/g×min) to 7.63 ml/g min at high flow rates (f = 2.55 ml/g×min)

Taking the mean capillary surface area S in the liver as ..50 cm /g (Crone 1963) the permeability coef ficient of ethanol P_{Eth}, can be estimated giving the mean value .83±1 05×10^{-4} cm × s (0.67<f<..55 ml/g × min^{-1}). The values of PS_{eth} in 7 expts with higher concentration of ethanol (addition of non radioactive ethanol) ranged from 3 6 ml/g × min at low rates (f=1 14 ml/g × min) to 6 15 ml/g × min at high flow rates (f = 15 ml/g×min) Mean P_{Eth} with higher ethanol concentration was 2.88±0.97×10^{-4} cm × s

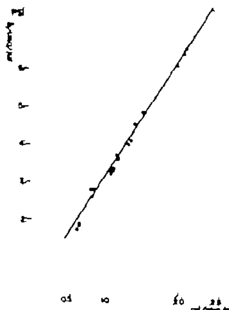


Fig 5 Effect of flow on capillary permeability of ethanol. PS_{eth} rises linearly s function of flow indicating flow limited distribution for ethanol in the liver Symbols are defined in the text.

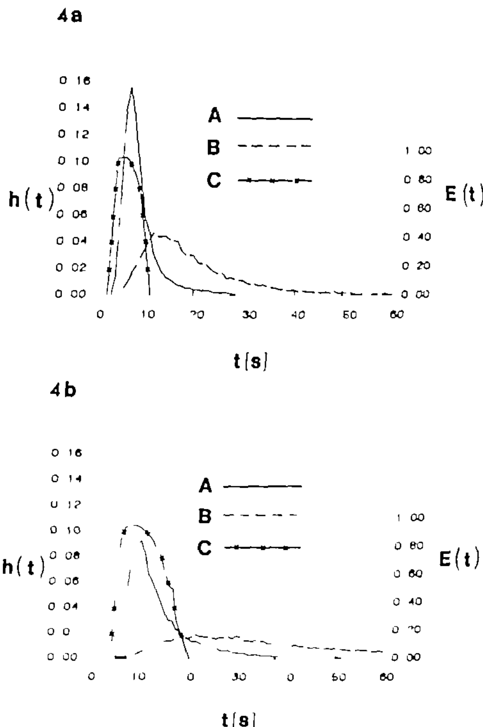


Fig 4 Time-activity curves of ¹C-ethanol (the diffusible test substance) and ⁹⁹ᵐTc-red blood cells (the non-diffusible reference substance) were recorded by continuous detection of radioactivity over the venous outflow. The transport function and the instantaneous extraction of ethanol are shown at two different flow rates f = 15 ml/g × min (Fig 4a) and f = 0.91 ml/g×min (Fig 4b). A ⁹⁹ᵐTc-red blood cells B C-ethanol C instantaneous extraction E(t)

outflow radioactivity pattern of the fast diffusible ethanol shows a lower maximal concentration and longer transit time than that of red cells, which reflects the different volumes in which ethanol and red blood cells distribute in the liver tissue. The instantaneous extraction of ethanol to the liver tissue is a fast process and the back diffusion of ethanol affects the descending part of the extraction curve. With equal values for h (t) and $h_0(t)$ the instantaneous extraction becomes zero

Liver weights, extractions and distribution volumes of ⁹⁹ᵐTc-red cell and C-ethanol in perfusion

Variables are explained in the text

meter	Liver weight (g)	E_{max}	V (%)	V (%)	?
riments without addition on radioactive ethanol[a]	13.4 ±0.3	0.95 0.01	18.7 ..5	56.2 9.0	09 0.75
riments with addition on radioactive ethanol[b]	13.4±0.3	0.95±0.01	19 6	57.2 9	2.04±0.54

perfusion expts on 7 animals.
erfusion expts on 3 animals.

f flow and concentration on ethanol action and capillary permeability— ce area product

experiments the flow of perfusion fluid ranged n 0.67 ml/g × min to 2.55 ml/g × min. In this ge E_{max} remained constant (0.95±0.01) indicat that flow does not influence the maximal ethanol raction (Table 1). The shortening of the plateau se of the instantaneous extraction curve (Fig.) can be explained by faster back diffusion of anol from liver tissue to intra ascular space with her flow rate.

In some experiments non radioactive ethanol was ded to the perfusion fluid to a final concentration 0.38±0.02%. This increase in the concentration ethanol did not change the maximal extraction lue (Table 1). The radioactivity of the liver was llowed by means of a separate gamma-ray onitor externally in 12 expts. to determine the nount of tracer which remained in the liver. The ean fraction of C-radioactivity remaining in the er after a single passage was 6% (range -8%) of e injected dose. At high flow rates (>2 ml/g × in) the fraction of C-radioactivity remaining in e liver was smaller (?%) than at low rates (<1 ml/g × min⁻¹ 8%). This fraction is not detected hen radioactivity is measured above the outflow bing. In calculations it should be taken into ac ount.

The relation between the flow rate and capillary ermeability surface area product is shown in Fig. Value of PS_{eth} increases linearly as a function of low rate (f). The equation describing the regression ne is $PS_{eth}=3.0±0.1×f($ = 0.98). The calculated PS_{eth} values ranged from 1.76 ml/g × min at low low rates (f 0.67 ml/g×min) to 7.63 ml/g× min at high flow rates (f=2.55 ml/g×min).

Taking the mean capillary surface area S in the liver as 250 cm /g (Crone 1963) the permeability coefficient of ethanol P_{EtOH} can be estimated giving the mean value ..83±1.05×10⁻⁴ cm × s (0.67<f<2.55 ml/g × min⁻¹). The values of PS_{eth} in 7 expts with higher concentration of ethanol (addition of non radioactive ethanol) ranged from 3.6 ml/g × min at low rates (f=1.14 ml/g × min) to 6.15 ml/g × min at high flow rates (f= 15 ml/g×min). Mean P_{EtOH} with higher ethanol concentration was 2.88±0.9?×10⁻⁴ cm × s.

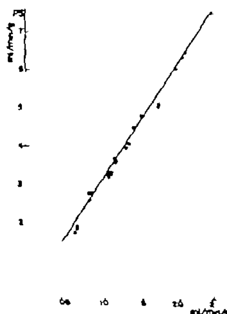


Fig. 3. Effect of flow on capillary permeability of ethanol PS_{eth} rises linearly as function of flow indicating flow limited distribution for ethanol in the liver. Symbols are defined in the text.

Volumes of distribution and distribution coefficient γ

The mean values and standard deviations of the distribution volumes for ethanol and red blood cells are presented in Table 1 The mean vascular distribution volume (V_1) calculated from the red blood cell curve was 18 7±2 5% of the total liver volume and the mean distribution volume of ethanol was 56 2±9 0% of the total liver volume in cases without addition of non radioactive ethanol The water content in isolated perfused livers after the experiment was determined in 3 expts and was found to be 67% (range 65–69%)

The distribution coefficient γ which describes the relative distribution volumes of ethanol and red blood cells was 2 1±0 7 It was used to test if some factor influences the distribution volume of the test substance The results indicate that variations of flow rate and ethanol concentration did not influence γ but the standard deviation was quite large

DISCUSSION

Extraction and capillary permeability—surface area product for ethanol

The purpose of this study was to investigate extraction and distribution kinetics of ethanol in the isolated perfused liver The viability of liver during perfusion experiment was investigated by determination of oxygen consumption A constant oxygen extraction for at least 90 min from the beginning of the experiment was found This agrees well with previous oxygen consumption measurements in the isolated perfused rat liver e g (Salaspuro 1968 Ross 1972) On the basis of these observations the perfusion technique used was considered to be valid The maximal extraction values describe the capacity of ethanol to penetrate the blood tissue barrier in the liver The small amount of C-methanol present in the C-ethanol preparation as impurity was considered to influence the results very little because methanol permeates capillaries about as effectively as ethanol (Raichle et al 1976) In the liver there are large intercellular fenestrations in the endothelial lining of capillaries between the sinusoidal cells and so all solutes crystalloids and colloids (M <90 000) may freely penetrate from intravascular space to the interstitial space of Disse (Bennet et al 1959 Keinänen et al 1979) The mean diameter of the space of Disse is only 2 μm (Goresky 1963) and the diffusion of a solute over this distance from the plasma ... liver cell may be considered instantaneous ... parenchymal cell membrane can be consid... be the main factor limiting permeability of e... in the liver Parameters extraction value ... PS_{cap} product measure therefore the permeab... of ethanol through the parenchymal cell memb... The obtained value for E_{max} for ethanol (0 9... agrees well with previous calculations in the ... Crone (1965) obtained 93% extraction and ... et al (1976) 97% extraction (f=0 5 ml/g × m... The accuracy of the instantaneous extraction ... ues is diminished because of the back diffus... fast diffusible ethanol which very soon beg... lower the extraction value (see Fig. 4 and Go... al 1975) Crone s model for capillary permea... in the tissues does not take into account back... fusion and to correct this we used E_{max} value... calculations of PS_{cap} values instead of the pe... traction E_p (Bassingthwaighte 1974) In more... plicated models of capillary permeability it is p... ble to separate the throughput component and... back diffusion component (double barrier mod... Goresky 1970) but their use is tedious becau... complicated mathematical formulas Other po... factors which can influence the instantaneo... traction value are the heterogeneity of ... blood flow and relatively faster axial intravas... diffusion of ethanol compared to red blood (Gullet et al 1975)

Since the extraction of ethanol is constant at ... ous flow rates in the liver and PS_{cap} is ... related to flow rate the ethanol distribution ... liver is flow limited as is the distribution of ... (Goresky 1963 Bravo & Yudilevich 1971 Ro... al 1977) The permeability coefficient P_i = (m... value 83×10 cm/s in experiments without ... dition of non radioactive ethanol) is comparable ... that previously calculated for labelled water in ... myocardial capillaries (6×10⁻ cm/s Rose et ... 1977) The permeability coefficient for labelled ... ter in the brain has been estimated to be 1 9×10 ... 3×10⁻⁴ cm/s (Eichling et al 1974 and Paul ... al 1977 respectively) P_{cap} calculated accordi... Crone s model is slightly underestimated beca... the model does not take into account the back ... fusion phenomenon The PS_{cap} value for brain ... ported by Raichle et al (1976) is of the same m... nitude (1 8 ml/g × min f=0 5 ml/g × min) ... PS for liver with same perfusion flow PS_{cap} ... ues without and with addition of non radioa...

ol differ slightly but the difference is so small
i may be considered to be insignificant

ne of distribution and distribution
k ent for ethanol

distribution olume of red blood cells in the (18.7± 5%) was similar to previous findings ←19% (Bra o & Yudilevich 1971) There is free technetium in the ^{99m}Tc-red blood cell arations (3.5% in our expts) and this pene- ← the space of Disse However the volume of space of Disse is a rather small fraction (3%) of total liver volume (Bravo & Yudilevich 1971 amen et al 1979) and therefore its influence on slight The ethanol distribution olume ±9 0% (ithout addition of non radioactive anol) agrees with the water distribution volume ver (6..% Bra o & Yudilevich 1971) and with calculations of the water content of the isolated used liver (67%) Investigation of the plot of C-ethanol curve on a semilogarithmic scale gests that the ethanol washout from the liver is biexponential which indicates that there are eral compartments into which ethanol pene es (space of Disse the liver parenchymal cell metabolized component) The fraction of the -radioactivity remaining in the liver after each ection (7–8%) causes some underestimation in calculation of ethanol distribution volume when ections are repeated during an experiment, be he mean transit time for ethanol decreases

The distribution coefficient γ is useful when the ect of certain factors on the distribution volume test substance is to be considered. I this study variations in either flow or ethanol concen- tion did not change ethanol distribution volume able 1)

Extraction of ethanol during a singl capillary ssage in the liver ha not been studied earlier sults obtained for the capillary permeability of hanol in the liver in this study are of same order as ose described earlier for the brain The increase the concentration of ethanol did not ha e any stinct effect on the permeability in the range used Distribution of ethanol in the perf sed liver was found to be flow limited Ethanol wa distributed to ater distribution volume The indicator diffusion chnique as shown to be useful in the study of nscapillary exchange of ethanol in tissues ^{14}C hanol has many advantages in the study of ethanol etabolism e g. regional distribution of ethanol can easily be followed by external detection without taking blood samples. Because of the short half-life of the tracer the experiments may be repeated fre quently The radiation dose is small which makes possible pharmacokinetic studies also in humans.

Authors are grateful to Prof M Bremer Ph D the head of the Åbo Akademi radionuclide project who organized the radiochemical work and provided the cyclotron of Åbo Akademi and the equipment of the Department of Physics, Åbo Akademi at our disposal We owe our warmest thanks to Prof E. Nordman, M.D and P Mäkelä, M.Sc who provided us with the equipment of the Radiation Therapy Clinic University Central Hospital Turku. Authors appreciate the collaboration and fruitful discus- sion with H Lundqvist Ph.D and M S Moller W want to express our gratitude to L. Hoverfält, M.Sc for con- structing electronic equipment and help in programming. Prof. Bremer Prof R. Saari M.D and Dr H Wendelin, M.D gave us valuable constructive criticism during the preparation of the manuscript. M Kerälinen is research fellow of the Finnish Academy The investigation was supported by a grant from the Finnish Foundation for Alcohol Studies, Helsinki.

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tension of pulmonary vein—left atrial junction art rate responses in conscious and anesthetized dogs

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NEVALAINEN T O HAKUMÄKI M O K HYODYNMAA S J NÄRHI M V O & SARAJAS H S S Distension of pulmonary vein-left atrial junction: Heart rate responses in conscious and anesthetized dogs. Acta Physiol Scand 1980 110 47-52. Received 23 Nov 1979 ISSN 0001-6772. Department of Physiology University of Kuopio and College of Veterinary Medicine Helsinki, Finland

The cardiac chronotropic effects of distension of pulmonary vein-left atrial junction were investigated in conscious dogs and in dogs anesthetized with intravenous alpha-chloralose (100 mg/kg) or pentobarbital (30 mg/kg). All the experiments were made on trained, chronically instrumented, closed chest animals held in horizontal position. Inflation of single small balloon in the junction elicited tachycardic response both in conscious and chloralose anesthetized dogs, while in pentobarbital anesthesia no change in heart rate was found Contrary to reports of other investigators no transient bradycardia was found nor any correlation between predistension heart rate and the increase in heart rate due to the distension. It is suggested that the choice of anesthetic can be done only after its modifying effect on the parameter studied is known Only the use of trained, chronically instrumented and conscious dogs will reveal this effect.

Key words. Mechanoreceptors, heart atrium, heart rate, consciousness, chloralose pentobarbital, dogs

reflex tachycardia has been found to result from distension of the pulmonary vein-left atrial junction in anesthetized dogs (Ledsome & Linden 4 Edis et al 1970 Kollai et al 1978, Chapman 1978). The afferent pathway of this reflex is served to be in the vagus (Ledsome & Linden 4) and the efferent pathway both in sympathetic in parasympathetic nerves (Kollai et al 1978 Chapman et al 1978)

Hitherto all the studies involving this reflex have been done on anesthetized animals, and all but one of the working groups have used open chest animals (Chapman et al. 1978). Yet there is growing evidence that anesthetics variously affect the cardiovascular functions and their neural control (Olmsted & Page 1966, Epstein et al 1968, Horwitz et al 1969 Sawyer et al. 1971 Vatner & Braunwald 5 Hahnen et al 1978) Furthermore opening of the thorax is known to influence the cardiovascular parameters including the cardiodynamics and the dimensions of the heart (Ferguson et al. 1953 Rushmer et al 1954 Morgan et al 1966 Giles & Burch 1970). To the best of our knowledge the effects of the distension of the pulmonary vein-left atrial junction have not been studied in conscious and closed chest animals. The present study was designed to compare the heart rate changes elicited by the distension in conscious dogs to those in dogs anesthetized with chloralose or pentobarbital

MATERIALS AND METHODS

Animals

Eight young laboratory-bred beagle dogs of both sexes, weighing 8–10 kg, were used in this study

Surgical procedure

Prior to the surgical procedure the dogs were accustomed to a restraining harness and laboratory surroundings during 3–4 consecutive days. A sterile left thoracotomy was performed under pentobarbital anesthesia and positive pressure ventilation. A thrombolectomy balloon catheter (capacity 2.5 ml) was passed via the pulmonary vein of the left apical lobe into the junction of the vein and left atrium

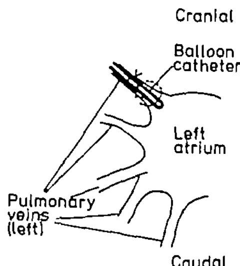


Fig 1 Dorsoventral silhouette of canine left atrium showing left pulmonary vein–left atrial junctions A thrombolectomy balloon catheter was passed via the pulmonary vein of the left apical lobe into the junction in such a manner that the tip of the catheter just entered left atrium Dotted line illustrates the silhouette of the inflated balloon

A more detailed location of the catheter is illustrated in Fig 1 The catheter was fixed in place with ligatures around the pulmonary vein and the left apical lobe of the lung was subsequently removed The catheter was passed out of the thorax and carried under the skin to the neck where it was placed under a protective collar The dogs were allowed to recover for 3–4 days after the surgery before the distension experiment

Experimental protocol

The distension experiments were performed on dogs fasted overnight and kept in restraining harnesses (Sine & Englert Jr 1965) in a natural horizontal position slightly off the floor The experiments took place in an isolated laboratory where external stimuli could be excluded The ECG signal was recorded telemetrically with needle electrodes and stored during the experiment on an instrumentation tape recorder (PI-6200). The distension period min when the balloon was inflated, was preceded followed by control periods of equal length (8 min conscious state the dogs were allowed to lay harness for about 10–15 min to calm down before commencement of the experiment Alpha-chloralose mg/kg % solution Alpha-D(+)-gluco-chloralose Merck) or pentobarbital (30 mg/kg Nembutal, was given intravenously via the cephalic vein prior to the experiment After experiments where the were anesthetized they were allowed to recover least 2 days before the next distension was commenced

Data processing

The heart rate was calculated for 15-s periods (HR_{15}) RR intervals of ECG

$$HR = \frac{\sum \frac{60s}{T_i}}{n}$$

Where n = number of complete RR-intervals during T = duration of individual RR interval (s)

The lengths of individual RR interval were measured by the timing unit of a multichannel analyser (Nokia 4900) with the accuracy of 1 ms. The heart rate change (ΔHR_{15}) was calculated for every 15-second period during the distension of the balloon and for the first 4 min after the deflation of the balloon The linear estimates of HR_{15} if no change had been induced for the periods mentioned above were calculated using the HR_{15} values of the last 4 min of the control periods both before and after the distension period This method of calculation is illustrated by an example in Fig 2. The aim of this procedure was to eliminate the changing trends in heart rate observed in a few of the experiments presumably due to the changing depth of anesthesia

The mean heart rate change and standard error of the mean were calculated from pooled data for every interval in conscious animal and those given chloralose or pentobarbital The statistical significance of changes was tested using the paired Student's t-test Furthermore the mean heart rate within ... min prior

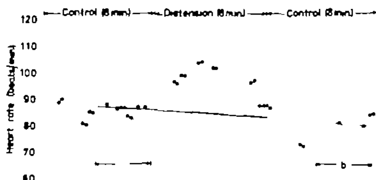


Fig 2 Illustration of the method used to calculate heart rate changes (ΔHR_{15}) in one experiment *The latter halves of both control periods (a and b) were used to calculate* linear estimates of HR_{15} (line) if no change had been induced Utilizing this estimate ΔHR_{15} values were processed for the distension period and the first half of the control period after distension

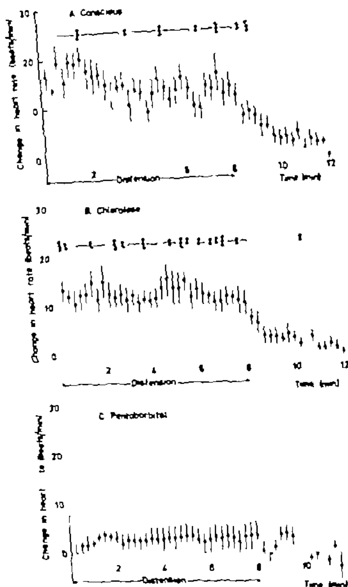


Fig. 3. Effect of distension of pulmonary vein–left atrial junction on heart rate change (ΔHR_{D}) in conscious dogs (A) and in those anaesthetized with alpha-chloralose (B) or pentobarbital (C). Vertical lines indicate standard error of mean (S.E.M.). Significance markings: *** $P<0.001$, ** $=P<0.01$ and * $P<0.05$. The predistension heart rates were 102.3 ± 18.6 per min (mean ± S.D.) for conscious, 87.5±7.8 per min for chloralose anaesthetized and 170.1±27.5 per min for pentobarbital anaesthetized dogs.

tension (initial heart rate) and the mean heart rate increase within 3 min after the distension of the balloon were tested for correlation.

RESULTS

Results from 25 distensions (average ΔHR_{D}) in conscious dogs are shown in Fig. 3 A. Distension of the pulmonary vein–left atrial junction elicited a tachycardic response which persisted through the whole distension period of 8 min. An increase in the heart rate was evident already during the first 15 s and maximal tachycardia was observed at 1 min after inflation of the balloon. The tachycardic response vanished within min after deflation of the

balloon The heart rate immediately before inflation of the balloon was 10? 3±18 6 per min (mean ± S D) in experiments with conscious animals The correlation coefficient calculated between the initial heart rate and the mean heart rate increase within the first 2 min after distension was 0 17 (n=25 N S)

Fig 3B presents the data from 8 distensions in dogs anesthetized with chloralose Again a tachycardic response resulted from the distension of the pulmonary vein–left atrial junction The response was observable within the first 15 s and *persisted all through the distension period After* deflation of the balloon the tachycardic response disappeared within 1 minute The dogs with chloralose anesthesia had a predistension heart rate of 87 5±7 8 per min (mean ± S D) The correlation coefficient calculated between the initial heart rate and the mean heart rate increase within the first 2 min after distension was 0 10 (n=8 N S)

In dogs anesthetized with pentobarbital the distension of the pulmonary vein–left atrial junction produced no significant changes in heart rate The results of 7 distensions under pentobarbital anesthesia are shown in Fig 3C The pentobarbitalized dogs had a heart rate of 170 1±?7.5 per min (mean ± S D) just prior to the distension The correlation coefficient calculated between the initial heart rate and the mean heart rate change within the first ? min after distension was 0 ?0 (n=7 N S)

DISCUSSION

The heart rate responses to distension of left atrial receptors are reportedly controversial Stimulation of these mechanoreceptors has been found to elicit *bradycardia (Daly et al 1937 Doutheil & Kramer* 1959) or to leave the heart rate unchanged (Daly et al 1937 Aviado & Schmidt 1959) According to Ledsome & Linden (1964) the overall response is tachycardia Consonant with this are the results of several subsequent groups (Edis et al 1970 Koizumi et al 1975 Chapman et al 1978 Kollai et al 1978) However even these more recent studies disagree among other things as to the initial effects of the distension on the heart rate and as to whether there is a correlation between the predistension heart rate and the degree of tachycardia attained

These controversial results may emanate from experimental variables *especially from the use and* depth of anesthesia *The most commonly* used anesthetics in physiological research are pe[ntobar]bital and chloralose However the impact of [these] compounds on the results and their interpreta[tion is] commonly disregarded This may be due to th[e fact] that the circulatory and autonomic effe[cts of] anesthetics are poorly known (Manders & V[atner] 1976) and that their direct and indirect effec[ts are] hard to differentiate from each other (Ha[...] 1977)

Pentobarbital is known to produce tachy[cardia] (Van Citters et al 1964) presumably owing [to its] vagolytic action (Morrison et al 1950 Vil[...] *Pardo 1967 Page & Hoff 1969) Furthermore* [spon]taneous and wide alterations in the rate-depe[ndent] variables are essentially eliminated by pentobar[bital] (Van Citters et al 1964)

Chloralose again is widely considered [the] anesthetic of choice in studies on circulato[ry con]trol Yet even this compound modulates the c[ar]diovascular and autonomic functions. It has be[en] reported to increase *the heart rate* and me[an] arterial pressure (Bass & Buckley 1966) F[ur]thermore it also disturbs autonomic reflex [ac]tions in an unpredictable fashion (Croft 1960) T[he] mechanoreceptor reflexes controlling the he[art] rate for example appear to be exaggerated [by] chloralose (Cox 197?)

To overcome complications produced by [anes]thesia we carried *out a series of distension*[s in] conscious animals According to our resul[ts a] tachycardic response to the distension of the p[ul]monary vein–left atrial junction can be elicite[d in] trained conscious dogs in circumstances where [ex]ternal stimuli have been minimized

When chloralose was used for anesthesia the[re] was a tachycardic response to the distension [...] *similar to that seen in* conscious dogs A dis[ten]sion induced tachycardia has been previously r[e]ported in dogs under chloralose anesthesia (Le[d]*some & Linden 1964 Edis et al 1970* Koizumi [et] al 1975 Kollai et al 1978 Chapman et al 19[78]) Only Edis et al (1970) and Kollai et al (1978) ha[ve] paid attention to the time course of heart ra[te] changes during the distension Edis et al report[ed] that tachycardia was the response to disten[sion] only in those dogs with an initial (predistension) heart rate below 140 per min whereas in those [with] initial heart rates over 140 per min there was [an] initial bradycardia lasting less than 1 min Su[b]sequently there was *no further change* Kollai [et] al in turn reported that the *stretch of the* [...]

-n-pulmonary vein region decreases the heart during the first 15 s following which a slight -ac acceleration supervenes. We were not able confirm these findings of Edis and Kollai's ps.

There was no correlation between the initial heart rate and the heart rate increments in our chloralose-anesthetized or conscious dogs. How- ever in neither group did we encounter initial heart rates as high as did Edis and Kollai's groups in their anesthetized animals. Actually in no experimental session of the present study did the initial heart rate come even close to the limit value of 140 per min. This difference may be traceable to the fact that we used plain chloralose in a somewhat higher dose than did the other groups, who besides this used morphine for premedication and thiopental for induction of anesthesia.

In this study we used 15 s intervals to calculate the heart rate. This interval should be long enough to eliminate the effect of sinus arrhythmia frequently encountered in dogs. If the initial bradycardic response to the distension was real, as suggested by Kollai et al. (1978), it should be seen in pooled data calculated for the first 15 s after inflation of the balloon (Fig. 3A for conscious and Fig. 3B for chloralose-anesthetized dogs). According to Kollai et al. the initial bradycardia occurred during the first 20 s after the stretch was begun. However as appears from Fig. 3A and B there was a highly significant increase in heart rate during the first 15 s in our conscious and chloralose dogs. Indeed, the screening through individual distensions shows that only one out of 25 distensions was there a decrease in heart rate in the conscious state while all distensions under chloralose anesthesia resulted in an increased heart rate at this interval. Kollai et al. stated that in experiments where only acceleration was observed large doses of chloralose were used. We used rather large doses of chloralose. Yet the fact that the initial cardiac deceleration was not detectable in our experiments is scarcely attributable to this. This is evidenced by the comparable heart rate responses in our conscious dogs free of any pharmacological intervention.

The discrepancy could be explained through the use of morphine which has been shown to have vagotonic effects even at small dose levels (Page & Hoff 1969). In the experiments presented here we did not use morphine as did the two other groups (Edis et al. 1970, Kollai et al. 1978). In dogs that had received only morphine premedication (mg/kg) distension failed to increase the heart rate (own unpublished observation).

In pentobarbitalized dogs distension of the pulmonary vein-left atrial junction did not have any effect on heart rate as shown in Fig. 3C. This could be explained through the vagal blocking effect of pentobarbital. Besides this vagolytic action, pentobarbital appears to have also sympatholytic properties (Halinen et al. 1978).

A wide variety of methods have been used to stimulate left atrial receptors. They include one to several balloons variously placed within or outside of the pulmonary vein-left atrial junction (Ledsome & Linden 1964, Edis et al. 1970, Kobzumi et al. 1975, Chapman et al. 1978). Others have used threads combined with a pulley and weights (Kollai et al. 1978). The use of several small balloons placed into the junction through pulmonary veins necessitates the ligation of all these veins (Ledsome & Linden 1964). This implies that practically no blood enters the left atrium from the left side of the lung.

A larger single balloon as used by Chapman et al. (1978) partially obstructs mitral valve and blood flow. The use of threads and pulleys necessitates the use of the open thorax. We used only one balloon placed in the junction of the left atrium and the pulmonary vein of the left apical lobe. This necessitated the removal of only one lobe, the apical, while preparing the animal. Our method of stimulating the receptors provides the least possible interference with blood flow with the possibility of using chronically instrumented closed chest animals. Our result also shows that one small balloon when correctly placed, produces sufficient stimulus for eliciting this cardioacceleratory reflex.

It appears that the distension of the pulmonary vein-left atrial junction evokes a tachycardic response, an effect that can be seen in unanesthetized conscious dogs and in dogs anesthetized with chloralose but not in dogs anesthetized with pentobarbital. No initial deceleration of the heart at the beginning of the distension was encountered. Nor was any correlation between the initial heart rate and the change in heart rate in any of the experimental states used.

We conclude that anesthetics should be used with care unless their modifying effect on the reflex studied is known. Apparently the quality of this effect as well as the effect of other corresponding

balloon The heart rate immediately before inflation of the balloon was 10? 3±18 6 per min (mean ± S D) in experiments with conscious animals The correlation coefficient calculated between the initial heart rate and the mean heart rate increase within the first ? min after distension was 0 17 (n=25 N S)

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In dogs anesthetized with pentobarbital the distension of the pulmonary vein–left atrial junction produced no significant changes in heart rate The results of 7 distensions under pentobarbital anesthesia are shown in Fig 3C The pentobarbitalized dogs had a heart rate of 170 1±27 5 per min (mean ± S D) just prior to the distension The correlation coefficient calculated between the initial heart rate and the mean heart rate change within the first ? min after distension was 0 ?0 (n=7 N S)

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When chloralose was used for anesthesia the was a tachycardic response to the distension similar to that seen in conscious dogs A dis sion induced tachycardia has been previously ported in dogs under chloralose anesthesia (Le some & Linden 1964 Edis et al 1970 Koizum al 1975 Kollai et al 1978 Chapman et al 19 Only Edis et al (1970) and Kollai et al (1978) h paid attention to the time course of heart changes during the distension Edis et al report that tachycardia was the response to distensi only in those dogs with an initial (predistensi heart rate below 140 per min whereas in those w initial heart rates over 140 per min there was initial bradycardia lasting less than 1 min S sequently there was no further change Kollai al in turn reported that the stretch of the l

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...sma water and ^{51}Cr EDTA equilibration volumes ...different tissues in the rat

...ARSSON L. JOHNSON G NYLANDER and U ÖHMAN

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LARSSON M JOHNSON L NYLANDER, G & ÖHMAN U Plasma water and ^{51}Cr EDTA equilibration volumes of different tissues in the rat. Acta Physiol Scand 1980 110.53–57 Received 28 Nov 1979 ISSN 0001-6772 Department of Surgery Karolinska Hospital, Stockholm, Sweden

The EDTA space and the plasma water olume of the whole animal and selected tissues were investigated with constant EDTA infusions of different duration in the rat The calculated EDTA space increased with the duration of the infusion both in the whole animal and the tissues sampled The plasma water volume remained constant, and therefore the calculated increase of the EDTA space suggests slow intracellular accumulation of the tracer As intracellular accumulation of EDTA increases with experimental time it is necessary to use as short an perimental interval as possible to reduce this error when determining extracellular fluid olumes

Key ords EDTA extracellular space isotopes, animal experiments

...e xtracellular fluid volume is measured by ...cer dilution techniques, where the marker dif ...ses throughout the extracellular *fluid* without ...grating into the cells. Such an ideal substance has ...t been found. EDTA has been chosen as a con...ient substance for studies of the extracellular ...id volume (Bernard & Biron 1971 Bohne et al ...66, Brading & Jones 1969 Virgilio et al 1970 ...lf et al 1971) It is almost completely eliminated ...rough the kidneys (Ahrens & Aronson 1971 ...rochner Mortensen et al 1969 Downes & ...AcDonald 1964 Foreman et al. 1953 Foreman & ...rujillo 1954 Garnett et al. 1967 Lachine et al ...376, Prpic 1967) with only an insignificant fraction ...eing metabolized (Volf et al 1971 Foreman et al ...953 Foreman & Trujill 1954 Havlicek et al ...968) or bound to plasma proteins (Bohne et al. ...968 Volf et al 1971 Garnett et al. 1967 Stacy & ...borburn 1966). We have recently investigated ...DTA tracer kinetics in the extracellular fluid in ...e rat (Larsson et al unpublished data). It was ...ound that the rapid renal elimination of the tracer ...r ubstance made space studies unreliable but when ...his renal loss was excluded the EDTA space could ...e determined with more precision. This latter mod...fication however involved surgical procedure ...hich in itself might bias the results.

The aim of the present study was to estimate the extracellular fluid volume and the plasma water olume of different tissues in the rat using Cr EDTA and ^{125}I human serum albumin as tracers. A constant nfusion of EDTA was used to compensate for renal losses and to achieve a stable concentration of the tracer To test the hypothesis of a slow but steady i tracellular penetration of EDTA we chose to vary the duration of the constant nfusion

METHODS

Male albino Sprague Dawley rats weighing approximately 250 g were used. They were kept in the laboratory under standardized conditions for at least one week before the expts. The animals fasted for 1... 18 h prior to the xpts, but received water ad libitum.

The rats were anesthetized with Pentothal Sodium (Abbott) intraperitoneally 5 0 mg/100 g b wt The left carotid artery and the right jugular vein were cannulated with fine bore catheters, the former for blood sampling and blood pressure registration (Siemens-Elema 746 pressur transducer and Siemens-Elema Mingograf 803 multichannel writer) the latter for tracer injections.

A commercial solution of ^{51}C EDTA in isotonic saline (Behringwerke AG Marburg) with concentration of 37 MBq/ml was used. Of this solution 0 1 ml/100 g b wt. was given as bolus dose followed by constant infusion (Syringe pump model 35.., Sage Instrument) with 35% of the bolus dose diluted to 1 ml and infused at rate of 1

factors operant during most acute experiments e g open chest side position and machined ventilation can be omitted only through the use of chronically instrumented trained and conscious experimental animals

This study was supported by the Academy of Finland and the North Savo Cultural Foundation

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e *Blood pressure (mmHg) plasma ater volume (ml/100 g b t) and EDTA space (ml/100 g b t) perimental group Mea ± S.D*

	Initial blood pressure	Final blood pressure	Plasma water volume	EDTA space
nin infusion	13 ±16	128_19	3 65_0 55	24 4±6 7
nin infusion	125_12	131_13	3 44 0 42	26 2±7.8
nin infusion	177 13	122 14	3 42 0 57	28 6_7.0 (60 min s. 120 min. $P<0\ 05$)

le 3 *Absol t nd l t method error in the determination of plasma water ol m nd EDTA spa g liver nd pleen*

	Lung		Liver		Spleen	
	Plasma water olume	EDTA space	Plasma water volume	EDTA space	Plasma water olume	EDTA space
mber of animals (3 samples per animal)	3	20	4	1	4	22
an (all samples), µl/g tissue	153	385	143	283	51	140
(all samples) µl/g tissue	33	59	37	56	6 2	77
olut method error µl/g tissue	14	79	15	18	_5	8.3
ative method error %	9_	7.5	10 5	6 4	4 9	5 9

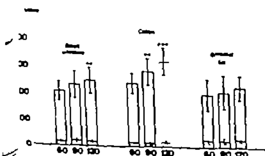
omparing the 120 min and 60 min groups, only lung showed a significant reduction in it plasma ater volume ($P<0\ 01$)

The 90 min values of the *EDTA space* for all the sues were greater than at 60 min although only liver ($P<0\ 05$) and the large intestine ($P<0\ 01$) ached statistical significance

At 120 min an increase of the EDTA space was ted for all the tissues except the skeletal muscle ling to reach significance only in the antral part of e stomach and the omental fat.

DISCUSSION

Intracellular accumulation of a tracer results in a higher calculated tracer space volume which is related to the experimental time The EDTA space of the experimental animals tended to increase with the length of the experiental time the 120 min value being 17% greater than the 60 min value A probable explanation is that intracellular accumulation of EDTA has occurred in proportion to the time of exposure



3 Plasma ater volume (hatched area) and EDTA ace of small intestine, colon and omental fat after DTA infusion of different duration Mean ± S D Sig- icance as in Fig 1



Fig 4 Plasma water volume (hatched area) and EDTA space of subcutaneous fat, skin and skeletal muscle after EDTA infusion of different duration Mean ± S.D Significance as in Fig 1

Table 1 *Number and body weight in the experimental groups Mean ± S D*

	n	Body weight (g)
60 min infusion	23	45±25
90 min infusion	1	61±39
120 min infusion	20	54±50

ml/h Infusions of 60 90 and 120 min were given Three min before the end of the infusion period a bolus dose of 0 1 ml/100 g b wt of an ¹²⁵I human serum albumin solution (130 kBq/ml) was given i v

Immediately following the end of the EDTA infusion the animals were sacrificed and the following tissues sampled lung liver spleen gastric fundus and antrum small and large intestine omental and subcutaneous fat skin and skeletal muscle The tissue specimens were immediately weighed wet and thereafter counted in a gamma scintillation detector (Selektronik model 54-__) From a representative number of experiments selected tissues were sampled in triplicate enabling the error of the method to be calculated

The following formula was used to calculate the tissue spaces of the tracers

$V = S_t/S_p$

where V = volume of distribution in tissue (µl/g) S_t = concentration of tracer in whole tissue (µg/g) S_p = concentration of tracer in plasma water (µg/µl) The plasma water volume was attained from the plasma volume by using the factor 0 929 (Altman & Dittmer 1961)

The EDTA space of the whole animal can be calculated from the biological half life of the tracer the rate of the constant infusion and the concentration of the tracer in plasma water in the steady state for EDTA that ensues when the renal losses of the tracer are balanced by the constant infusion (Moore et al 1968) The following formula was used

$V = (I \times T_{\frac{1}{2}} \times 1\ 44)/Sp$

where V = EDTA space of the whole animal I = intake (=output) of the tracer $T_{\frac{1}{2}}$ = biological half life of the tracer Sp = concentration of tracer in plasma water

Conventional statistical methods and tables (Armitage 1971) were used Inter-group and intra-group mean values were compared according to Student's *t* test for independent and dependent observations. When the variance differed considerably the mean values were compared using the *t* test described by Welch (Welch 1947) The method errors were calculated with analysis of variance Values are given as mean and the standard deviation

RESULTS

Table 1 gives the number and body weight of the animals in the different experimental groups

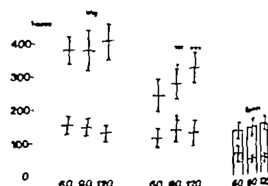


Fig 1 Plasma water volume (hatched area) and EDTA space of lung liver and spleen after EDTA infusion of different duration *Mean ± S D* Statistically significant differences are indicated (P<0 05) (P<0 01) (P<0 001).

In Table 2 initial and final blood pressure plasma water volume and EDTA space at the end of the experimental period are presented The blood pressure values and the plasma water volumes in each group were the same while the EDTA space increased with the duration of the infusion time The difference was significant between the 60 min and the 120 min groups

In Table 3 the error of the method for estimating the volumes of plasma water and EDTA space in selected tissues is presented

In Figs 1–4 histograms are presented showing the plasma water and EDTA equilibration volumes for all the tissues Compared with the 60 min group the *plasma water volume* tended to decrease in most of the tissues after 90 min EDTA infusion This decrease was however significant only in the spleen (P<0 02) the large intestine (P<0 05) and skeletal muscle (P<0 02) The liver however increased its plasma water volume (P<0 05).



Fig 2 Plasma water volume (hatched area) and EDTA space of fundus and antrum of the stomach after EDTA infusion of different duration Mean ± S D Significances as in Fig 1

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The plasma water volume of the whole animal was unaffected by the length of experimental time and gave consistently normal results in all three groups (de Boer et al 1975)

The results concerning the plasma water volume of the tissues should be regarded with some caution *since variations could be attributed in part to a* variable blood loss during the process of dissection (Everett et al 1956) The determination of the plasma water volume serves in this study mainly as a control to answer the following question Is any observed change of the EDTA space due to a change of the plasma water volume and thus possibly an artifact?

The estimation of the method error shows that only a small degree of the total variance in the calculation of plasma water and EDTA equilibration volumes depends on imperfect tissue sampling sample weighing and isotope counting However the differences between tissue samples from the same organ of the same animal contribute a greater part of the total variance while the major portion is due to the biological variation between animals

Relating the volume of plasma water or EDTA space to the wet weight of the tissue sample causes that any observed change in comparison to the control value will be calculated as a lower figure than the real one assuming no concomitant change in the other fluid volumes If for example the EDTA space decreases from 250 to 200 µl/g tissue (real reduction) no change occurring in other fluid compartments the calculated reduction of the EDTA space will be from 250 (=250 µl/1 000 mg tissue) to 211 µl/g tissue (=200 µl/950 mg tissue)

The EDTA space in different tissues showed a uniform tendency to increase with the duration of the EDTA infusion The increase at 90 min compared to the 60 min values was 14% in the liver and 18% in the large intestine After 120 min infusion the enlargement of the EDTA space was 34% in the liver 22% in the gastric fundus 33% in the large intestine ($P<0.001$) 18% in the small intestine 30% in the skin ($P<0.01$) 10% in the lung 13% in the spleen and 17% in the subcutaneous fat ($P<0.05$) The increments of the EDTA space can not be explained by changes in the plasma water volume

The enlargement of the EDTA space in the liver is of particular interest since this could be due to EDTA excretion in the bile No attempt was made in these experiments to test this theory If biliary excretion of EDTA occurs it is in neg amounts but has been denied by most a (Bröchner Mortensen et al 1969 Downes McDonald 1964 Foreman et al 1953 Garnet 1967 Prpic 1967 Havlicek et al 1968, Lakg Pedersen & Engell 1972)

Both determinations of the total EDTA sp the animal and the EDTA space of different ti suggest that the tracer penetrates into the intra lar fluid space Thus when using EDTA as a terminant of changes in the extracellular space it is necessary to minimize and standa the length of the infusion time in the experim animals and their controls

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hysiol Scand 1980 110: 59–67

traction of the rat portal vein in hypertonic isotonic medium mechanical properties and cts of Mg^{2+}

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HELLSTRAND P & ARNER A. Contraction of the rat portal vein in hypertonic and isotonic medium: mechanical properties and effect of Mg^{2+}. Acta Physiol Scand 1980, 110 59–67. Received 4 Dec 1979. ISSN 0001-6772. Department of Physiology and Biophysics, University of Lund Sweden.

The contracture elicited in the rat portal vein by hyperosmotic solutions (HC) has been investigated with respect to its Mg^{2+}-dependence and its mechanical properties. Comparison was made with K^+-induced contractures (KC) of similar force. In contrast to its relative resistance to Ca^{2+}-depletion, HC is entirely abolished after depletion of both Ca^{2+} and Mg^{2+} ions. After readdition of Mg^{2+} alone HC can be partially restored again. Isotonic quick release expts were performed on muscles in HC, KC and after passive stretch (PS) to a similar total tension and the length responses following the releases were analyzed. The immediate elastic response showed that stiffness was markedly higher in HC than in KC, whereas in PS it was much lower. After the elastic response a quickly decaying exponential component could be distinguished from the later more steady length change. The time constant of this transient response was 40–50 ms (KC) or 25–30 ms (HC). On release to a minimal afterload the amplitude of the transient response was about 1% (KC) or 0.5% (HC) of the initial muscle length, decreasing with increasing afterload. A corresponding transient lengthening response was seen after a step to a force greater than the isometric tension. Transient length responses in PS were much smaller than in KC or HC following force steps producing similar elastic recoils. The late shortening in HC and KC, interpreted as the isotonic response of the contractile system, could be fitted to Hill's eq. V_{max} in KC ($[Ca^{2+}]$=0.5 mM) was 0.25 ± 0.02 and in HC 0.05 ± 0.01 (SE, 6) lengths/s. The results of this investigation support the conclusion that HC originates in the contractile system of the muscle. The quantitative differences in mechanical properties are discussed against possible alterations in HC of cross-bridge behaviour and/or filament structure.

Key words: Smooth muscle, hyperosmolality, Mg^{2+}, contraction, mechanical transients, elastic recoil, force–velocity relation

sustained tension increase of the rat portal vein in response to hyperosmotic solutions has been described and was shown to be largely independent of external Ca^{2+} supply (Andersson et al. 1974). However the response was found to be greatly diminished at low temperature and during metabolic depletion, suggesting that it is due to activation of the contractile system of the muscle. The mechanical behaviour of the portal vein in hyperosmotic solution indicated a greater stiffness and a lower shortening speed than in a K^+-induced contracture of similar force. In the present study the ionic dependence and mechanical properties have been further characterized, including an analysis of the transient length changes occurring in the first few milliseconds after a force step (Johansson, Hellstrand & Uvelius 1978, Hellstrand & Johansson 1979). The results indicate a marked Mg^{2+}-dependence of the hyperosmolality contracture, providing further evidence that it is caused by activation of the contractile proteins of the smooth muscle. There are however large quantitative differences in mechanical properties between the hyperosmolality contracture and the K^+-induced contracture, suggesting that the contractile system may be in quite different states.

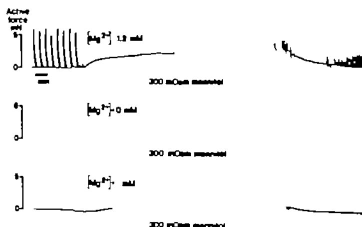


Fig. 1 Upper panel response of portal vein to hyperosmolal solution 300 mOsm mannitol added to normal medium. Middle panel: response after 60 min exposure to Ca^{2+}-free, Mg^{2+}-free solution containing 1 mM EDTA Lower panel: response 15 min after addition of 5 mM Mg^{2+}

der to effectively deplete intracellular Mg^{2+} it is necessary to expose the muscle to a ion free of both Mg^{2+} ions and Ca^{2+} ions rdsson & Uvelius 1977). In Fig. 1 the upper shows control resonse of a portal vein in a ion of normal ionic composition when osmola- as increased by the addition of 300 mOsm/kg itol The hyperosmotic solution was then re d by one of normal osmolality lacking added and Mg^{2+} and with 1 mM EDTA present to te trace amounts of these ions. 60 min later the rosmolality response was completely absent lle panel). On exposure to the hyperosmotic ion 15 min after the addition of 5 mM Mg^{2+} ver a weak contracture appeared (lower pan- Note that no Ca^{2+} ions were added addition of (5 mM) alone with no Mg^{2+} present was not to restore the HC after divalent cation deple After return to normal medium the muscle re d spontaneous activity although this was of frequency and low amplitude The Mg^{2+} sen- ty of HC was consistently seen in 1 expts

istic response

ne quick release expt were performed to cterize the dynamic behaviour of the portal in HC as compared to KC and to condition of ased passive tension, produced by stretch Some data on the force–velocity relation and tistic properties were reported by Andersson (1974), but the present expts have been per ed with much improved time resolution, allow- ing steady afterload after a force step to be established in a few ms (Sjolin et al 1978) Fig 2 shows superimposed representative length responses in KC HC and PS The relative force steps P/P were

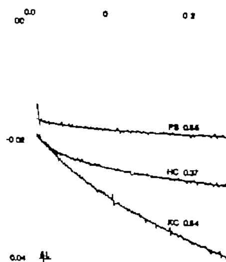


Fig 2 Superimposed length responses to force steps in portal vein. Records were digitized at 1 kHz and displayed together with double-exponential fit (broken lines). Downward deflection marks shortening Relative force step given by number beside each tracing Upper tracing passive stretched muscle (initial tension 3 1 mN length 6.3 mm, weight 2 mg), middle tracing hyperosmolality contracture (initial tension 3 6 mN length 4 4 mm), lower tracing K -induced contracture (initial tension ..7 mN length 4 4 mm). Length responses normalized to the short er length.

In a separate paper (Arner & Hellstrand 1980) the metabolic flux rates associated with responses to hyperosmolal or K high solutions are reported

METHODS

Portal veins were dissected from male Sprague-Dawley rats (200–400 g) and cleaned of fat and adventitial connective tissue They were mounted and left to accommodate for at least 45 min in a solution ("normal tris") of the following composition in mM NaCl 1?0 KCl 6 0 $MgCl_2$ 1.2 CaCl ?.5 glucose 11 5 Na_2Ca-versenate 0 026 and tris (hydroxymethyl)-aminomethane (Trizma Base Sigma Chemical Co) 23 at pH 7 4 bubbled with 100% O_2. Nominally Ca free tris solution was prepared as normal tris except that Ca^2 was omitted Temperature was kept at 37°C throughout the expts Contraction was elicited either by a solution where 100 mM NaCl of the normal tris had been replaced by KCl (" K high tris) or by a solution made hyperosmotic by the addition of mannitol to the normal tris To make these two kinds of contraction similar in magnitude it was necessary to use a relatively low [Ca] (0.5 mM) in the K high tris. Some expts were performed in Mg^2 and/or Ca^2-free solutions containing EDTA (1 mM) to chelate the divalent cations Osmolality of the solutions used was routinely checked on a Advanced Instruments Inc freezing point osmometer (31–LAS) and amounted to about ?90 mosm/kg H_2O in the normal medium and 590 mosm/kg H_2O n the hypertonic solution Two kinds of mechanical expts were performed (i) isometric recording in a 50 ml organ bath using Grass FT03 force transducers (ii) isotonic quick release expts using the apparatus described by Sjolin Hellstrand & Clementz (1978) In the latter expts the force and length records were stored on magnetic tape and subsequently digitized at a sampling frequency of 1 kHz for computer analysis (Hellstrand & Johansson 1979) The program performs a f t of the length record following the initial elastic recoil to a model consisting of a sum of two decaying exponential functions

$$L(t) = L + A (1-e^{\ \ }) + A_2(1-e^{\ \ }) \qquad (1)$$

Here L is the muscle length at t=0 after the end of the elastic reco? A and τ are the ampl tude and time constants respectively of a fast" (i=1) and a slow (i=2) exponential phase For shortening of the muscle $A_i<0$ and for lengthening $A_i>0$ In such an analysis performed on rabbit bladder smooth muscle (Hell trand & Johansson 1979) the fast and slow phases could be disti guished by different dependence on the level of act vation of the muscle whereas the temperature dependence was found to be similar

The shortening velocity at 100 ms after release was obtained by differentiation from the double exponential fit This was used for construction of force velocity curve by a computer fit to the Hill equation

$$V = b(P - P)/(P + a) \qquad ( )$$

The series elastic (SE) recoil were determ[ined] intersection of the fitted double-exponential or the length record during the elastic respon[se] were fitted to a logarithmic curve

$$\frac{\Delta L}{L} = \frac{1}{k} \times \ln \frac{P-B}{A}$$

where A B and k are constants and L is the ... length In construction of SE force-extension ... cords with $P>P_o$ as well as P<P ... force–velocity curves computed according to eq data with P<P were used

In the quick release expts the muscle was first normal tris at a low preload (<0 75 mN) Expts ... tonic and K high solutions were then perform[ed] ing with hypertonic solution in 3 of the expts ... K high in the 3 others K -contractures (KC) ... duced by repeatedly exposing the muscle to K ... ution for 1 min followed by normal tris (0.5 mM ... min When reproducible contractions had ... lished quick releases to varying afterloads (P<P) performed in a random order In some of the expts stretches ($P>P_o$) in order of rising afterload were after all the releases had been made In the hyperos[molali]ty contractures (HC) all releases and stretches were while the muscle remained contracted No loss of ... tility was seen during this procedure Between the runs the muscle was allowed to recover for at least ... in normal tris (2.5 mM Ca^2)

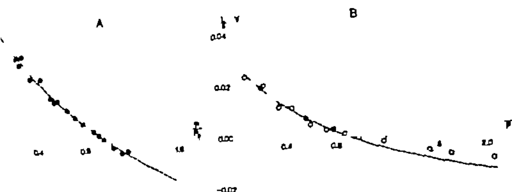
After the HC and KC responses had been deter[mined] muscle wa relaxed in nominally Ca^2-free ... normal osmolality It was then stretched until its tension equalled the total force reached in the ... sponses, and kept at this new length A series of ... and stretches of the relaxed muscle was then record[ed in a s]imilar way as before

At the end of the expt the muscle was returned ... original length which was determined by microscopy an ocular scale It was then removed, blotted weighed on a Cahn electrobalance Cross sectional wa computed from the length and wet weight ... uniform thicknes and a density of 1 05 mg/mm³

RESULTS

1 Mg^2-dependence f the hyperosmolality contracture (HC)

The contracture elicited in the portal vein by ... ly hyperosmotic solutions persists even if ext[ernal] [Ca] is reduced drastically below the limit for ... traction evoked by other stimuli (Andersson e[t al.] 1974) We observed however that the hyp[er]osmolality contracture (HC) is critically dependent [on] Mg^2 ions and in that respect resembles other ... of active contraction in the smooth muscle ... omitting Mg^2 from the external solution does ... affect either HC or K induced contractures



Force elocity relations in K -contracture (A) and in hyperosmolality contracture (B) Same expt as in Fig. 3 ities measured 100 ms after release. Note different scales on velocity axis)

KC open circles and broken line from HC, rosses and full line from PS Parameters of the ure given in the legend It is evident that the le is stiffer in HC than in either KC or PS A parison of mechanical parameters in HC and rom 6 muscles is shown in Table 1 It is seen the dimensionless constant k from eq (3), ch characterizes the stiffnes of an exponential ing, is significantly greater in HC despite similar ors of developed stress.

Force- locity relation

shortening record in the first 50–100 ms follow he elastic response is seen from Fig. 2 to be racterized, in the cases of KC and HC, by a lly declining elocity as shortening proceeds. such beha iour is seen also in PS but the cle here beha es essentially as passive elastic . The phenomenon of rapidly diminishing short g velocity may be generally occurring in oth muscle but can be analyzed only in ex riments made with sufficient time resolution arrison, Hellstrand & U elius 1978 Hellstrand ohansson 1979 Mulvany 1979) To specify the e-velocity relation it is necessary to fix a point me after the release at which the velocity is to measured. In this and earlier work we have sured velocity at 100 ms after release since the ent rapid shortening has essentially then and th velocity may be considered characteristic of the steady state" behaviour of the contractile system (Hellstrand & Johansson 1979)

Representative force-velocity records at 100 ms in KC and HC are shown in Fig. 4 A and B respectively It is seen that shortening, as well as lengthen-

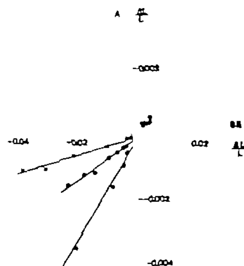


Fig 5 Amplitude A_1 of exponential isotonic transient [cf eq. (11)], lotted against magnitude of immediate elastic recoil (SE). Same expt as in Fig. 3 Open circles hyperosmolality contracture Filled circles K -contracture Crosses, passive stretched muscle

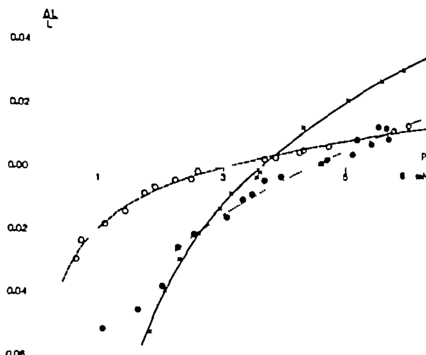


Fig 3 Force-extension relations of initial elastic response Logarithmic fit to eq (1) shown Data from one muscle Open circles broken line hyperosmolality contracture (muscle length 6 8 mm weight 1 95 mg parameters of fit k=69.5 A= 84 mN B 0 28 mN) Filled circles dotted line K -contracture (length 6.8 mm k=35 0 A=4.50 mN B=−0 5 mN) Crosses full line passive muscle (length 9 mm k=29 6 A=1.84 mN B=1.58 mN) In the passive muscle length responses were normalized to the shorter muscle length of the other two plots, to allow comparison of absolute magnitudes

chosen to give elastic recoils of similar magnitudes and are shown by the number beside each tracing The output from the length transducer was digitized at 1 kHz and then displayed graphically Superim posed on the length tracings are the double-exponential fits calculated according to eq (1) (see Methods) In HC and KC the preload on the muscle was kept low 0 6 mN to minimize influence of parallel elasticity whereas in PS the passive muscle in Ca free solution had been extended by 43 % of its original length to produce a force of 3 1

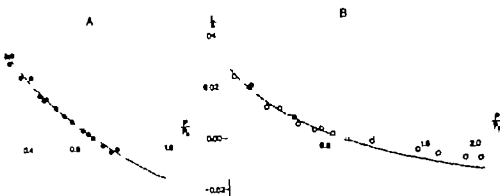
Table 1 *Mechanical parameters of portal veins in KC and HC*

Mean values ± S E of 6 expts P_0, a and b defined by eq ( ) $V_{max} = bP_0$ The dimensionless stiffness parameter k defined by eq (3) $P<0.01$ $P<0.001$ by paired Student s *t* test

		K -contr	Hyperosm
P_0	mN/mm²	9 9±1.3	9 8±0 8
k	–	34±3	55±5
V_{max}	lengths/s	0 .3±0 0.	0 05±0 01
a/P_0	–	3 0±0 6	7±1 4
b	lengths/s	0 68±0 1	0 07±0 04

mN It is seen that although the elastic recoils about the same magnitude the force step prod this recoil is the greatest in HC smaller in KC substantially smaller in PS Thus the stiffness greatest in the HC response Clearly due to rather small active tension developed by HC the fact that [Ca^{2+}] was kept as low as 0.5 in KC to produce similar active tension parallel ticity cannot strictly be neglected in these either However no attempt has been made to rect for this since such a correction is likely very unreliable and depends critically on the sumptions made as to the geometrical arrange of the functional mechanical elements in the cle The comparisons made here must there necessarily be regarded as qualitative Emphasis placed on the comparison of HC and KC since are at the same total length of the muscle and sumably therefore at the same level of tension the passive elements

In Fig. 3 are shown logarithmic fits according eq (3) made to the elastic responses to force st of different magnitudes All data are obtained f one muscle Filled circles and dotted line show



Force elocity relations in K -contracture (A) and in hyperosmolality contracture (B). Same expt as in Fig 3 es measured 100 ms after release. Note different scales on velocity axis

C open circles and broken line from HC ases and full line from PS Parameters of the given in the legend It is evident that the is stiffer in HC than in either KC or PS. A rison of mechanical parameters in HC and om 6 muscles is shown in Table 1 It is seen he dimensionless constant k from eq (3) characterizes the stiffness of an exponential is significantly greater in HC, despite similar of developed stress

rce–velocity relation

hortening record in the first 50–100 ms followe elastic response is seen from Fig. to be cterized in the cases of KC and HC by a ly declining elocity as shortening proceeds. e such behaviour is seen also in PS but the le here behaves essentially as a passive elastic

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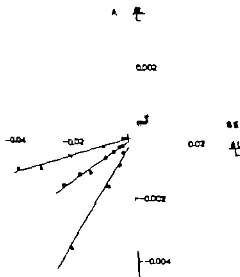


Fig 5 Amplitude A_1 of exponential isotonic transient [cf eq (1)], plotted against magnitude of immediate elastic recoil (SE). Same expt as in Fig. 3 Open circles hyperosmolality contracture Filled circles, K -contracture. Crosses, passive stretched muscle

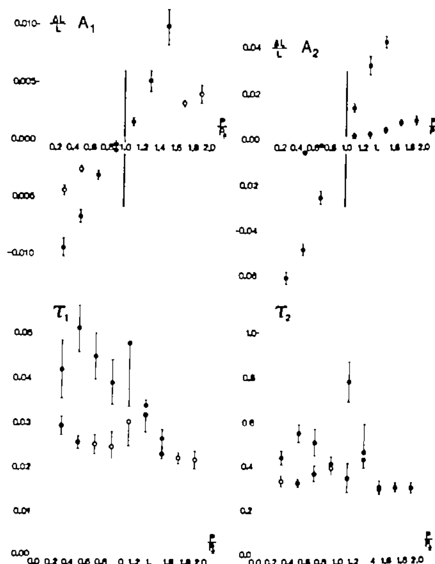


Fig 6 Amplitudes (A A) and time constants ( ) of exponential components in fits to length records according to eq (1) Filled circles K -contracture Open circles hyperosmolality contracture Data from 6 expt grouped according to P/P into classes with a width of 0 2 Mean values ± S E shown Each point represents 3–16 values. Broken lines emphasize transition between shortening and lengthening responses.

ing velocities are considerably lower in HC (note the difference in scale) The hyperbolae are fitted to the points with P<P according to Hill s eq (?) Force–velocity points for P>P at or above the continuation of the hyperbola were seen in 5 expts out of 6 in HC whereas in KC they consistently fell below the hyperbola (cf Johansson et al 1978)

The constants characterizing the force–velocity relations (eq ?) a/P b and maximal shortening velocity $V_{max}=b\times P_o/a$ found in the 6 expts are shown in Table 1 (mean values ±S E) Both V_{max} and b are considerably lower in HC the difference being highly significant Values for a/P are not significantly different They are rather high and spread considerably (cf Hellstrand 1979) Th[illegible] be a consequence of the relatively small devel[illegible] tension as compared to preload in the present [illegible]

4 *Transient is t al respo nses*

The time-dependent response to a force [illegible] ("isotonic transient) with rapidly diminish[illegible] shortening (or lengthening) velocity was anal[illegible] in a manner analogous to that used on rabbit [illegible] muscle by Hellstrand & Johansson (1979). The [illegible] proach is to treat the length record as the su[illegible] two exponential components (eq (1)) each c[illegible] acterized by its amplitude and time constant ([illegible] Methods) Isotonic transients were seen in the f[illegible]

ts on the portal vein both in KC and in HC was also a phenomenon of similar time scale onstant 20–50 ms) in the releases made in PS responses, particularly at low afterloads, however not be fitted with certainty to the xponential model due to prominent inertial tions

5 (data from 1 expt) shows that relative to the recoil (SE), the amplitude (A_1) of the isotonic nt is largest in KC smaller in HC and smaller PS

g 6 are plotted the amplitudes A and A_2 and ne constants and τ for the HC and KC nses Data from 6 expts were grouped into according to P/P_o. PS responses are not ed mc fits could not be obtained with cer and since ambiguities of interpretation may hue to the different muscle lengths (note that not a problem in Fig. 5 where both axes are lated to muscle length) The amplitudes A of t xponential components is smaller in absomagnitude in HC than in KC both for shortend lengthening responses. The time constant shortening responses, smaller in HC than in The slow exponential component had considsmaller amplitude (A_2) in HC than in KC but w time constant ( ) This corresponds to the n velocity n KC as already seen.

the stretch responses (to the right of the m lines in the diagrams) the amplitudes A and ntinue smoothly from the shortening side as the time constants show considerable scat r small force steps They generally seem to e with greater force step, but the tendency is and cannot be judged w th certainty Note here is a difference of an order of magnitude een and implying that the separation into processes is quite reliable

CUSSION

hyperosmolality contracture (HC) was found to ependent on extracellular Mg^2 ions to the same al a the spontaneou activity or the contrac elicited in the portal ein by K -high medium Sigurdsson & U ehus 1977) This is in contrast (relative resistance to Ca^{2+}-depletion (Anson et al 1974). E idence has been presented HC is an active response of the muscle rather consequence of passive cell shrinkage (Anson et al. 1974 McGrath & Shepherd 1976)

The Mg^2 -dependence of HC suggests that Mg^2 dependent (actomyosin) ATPase is in ol ed in the response

The low sensitivity of HC to Ca^2 depletion might indicate either that in the hyperosmotic solution Ca^{2+} ions are released from intracellular stores or that the contractile system becomes sensitive to very low Ca^2 levels or altogether independent of Ca^{2+} regulation. It is interesting that in chemically skinned smooth muscle cells a moderate elevation of the Mg^2 concentration of the medium is able to elicit contraction even in the absence of Ca^2 ions (Saida & Nonomura 1978) Ca^{2+}-independent ATPase activity occurs in regulated actomyosin systems from skeletal muscle in vitro when the level of ATP is low (Weber & Murray 1973). This phenomenon is attributed to the presence of some attached rigor complexes removing the inhibition of the active sites normally effected by the tropomyosin molecule in the absence of Ca^{2+} ions No evidence for such a mechanism in smooth muscle has however been presented, to our knowledge

Although activation by Ca^2 probably accounts at least in part for HC in the portal vein there remains the possibility that Mg^2 -dependent ATPase activity is initiated in the hyperosmotic response independent of Ca^2 regulation

The mechanical expts on the portal vein in hyperosmotic solution indicate an increased stiffness, both with respect to the relaxed state in normal solution and to the K -induced contracture (KC Fig. 3) In addition, the shortening velocity is greatly reduced compared to that in KC (Fig. 4 and Table 1) Qualitatively these effects were noted earlier (Andersson et al. 1974). The isotonic transient responses seen in the portal vein and other smooth muscles (Johansson et al 1978 Mulvany 1979) have tentatively been interpreted in terms of a cross-bridge mechanism of contraction (Hellstrand & Johansson 1979) In the present study it is shown that both the amplitude (A) and the time constant (τ) of the exponentially decaying isotonic transient length response are reduced in HC as compared to a KC of similar force (Fig. 5 and 6)

Phenomenologically the transient mechanical responses in smooth muscle may be thought to result from an undamped and a damped elasticity connected in series in a Voigt element (Mulvany 1979) In terms of this model the present results would mean that both springs have become stiffer in the hyperosmotic solution although not proportionally

so as shown by Fig 5 In a linear Voigt model the time constant τ_1 would be determined by the quotient viscosity/stiffness of the damped element If a linear approximation is applicable the results in Fig 6 (τ_1) would mean that in HC thi quotient is *reduced* The active shortening subsequent to the transient responses after a force step is not explained by the viscoelastic model but requires the presence of an independent force or shortening generator (cf Mulvany 1979) The greatly reduced *shortening velocity in HC* compared to KC (Fig 4 A and B) will thus mean that the generator has profoundly altered its properties in the hyperosmotic environment

Differences in shortening velocity and metabolic demand of contraction between different kinds of muscle have been considered against differences in the length of the contractile units (*sarcomere* analogues) of the muscles (Rüegg 1971) Thus in muscles with longer contractile units shortening velocity would be smaller at equal rate of turnover of cross bridges One would also predict that length transients representing relaxation phenomena *within the individual sarcomere analogues* would have a smaller overall amplitude in a muscle where these units are longer The thick filaments in taenia coli smooth muscle were suggested by Lowy & Small (1979) to exist in the form of long ribbon like structures However Somlyo Somlyo & Devine (1971) showed in expts on the rabbit portal vein that ribbons were produced consistently when the muscles were stretched and/or treated by hyperosmotic solutions similar to those used by us whereas round filaments were seen at normal tonicity In subsequent expts the length of these round filaments were determined to be about 2 2 µm (Ashton Somlyo & Somlyo 1975) thus somewhat longer than the thick filaments of skeletal muscle It is an interesting possibility that the morphological characteristics of portal veins in hyperosmotic solution may be related to the mechanical properties reported here Thus if ribbons are present it i conceivable that the contractile units of the muscle functionally behave as if their length had been considerably increased It is entirely plausible however that the hyperosmotic solution will cause biochemical alterations in the muscle affecting the rate and extent of cross bridge turnover Expts relating to the metabolic demands of contraction in KC and HC are reported in the concomitant paper (Arner & Hellstrand 1980) They indicate that in hyperosmotic medium the cross-bridge cycle operates essentially normal way but that the number of active cross bridges may be reduced This suggest that the increased stiffness and lower in hypertonicity are caused by some factors reducing internal resistance to length change.

We would like to thank Monica Lundahl Heidenholm and Ina Nordstrom for technical assistance The investigation was supported by grants from Swedish Medical Research Council (04X-...) Medical Faculty University of Lund and ... Göteborg

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Physiol Scand 1980 110 69–75

ntraction of the rat portal vein in hypertonic d isotonic medium rates of metabolism

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ARNER, A & HELLSTRAND P Contraction of the rat portal ein in hypertonic and isotonic medium ates of metabolism. Acta Physiol Scand 1980, 110 69–75 Received 4 Dec 1979 ISSN-6772. Department of Physiology and Biophysics University of Lund S eden.

The contracture induced by hyperosmolality (HC) in the rat portal vein has been investigated with respect to it metabolic properties. Oxygen consumption (J_{O_2}) and lactate production (J_{LA}) ere measured Comparison was made with K -induced contractures (KC). Variation in active force was produced for both kind of contracture by alteration of muscle length. Measurements were also made in normal isotonic solution, where the muscle is spontaneously activ and in Ca^{2+}-free isotonic solution. At short muscle lengths here no activ tension was produced J_{O_2} was lower in HC than in KC I contrast, J_{LA} was greatly enhanced in HC so that calculated ATP turnover (J_{ATP}) was almost the same in both kinds of solution Comparison of metabolic rates at long and short muscle length and correlation to force development allowed calculation of tension-dependent metabolism. In normal solution (spontaneous activity) tension-dependent metabolic rate was much greater than in either kind of contracture No difference in tension-dependent J_{O_2}, J_{LA} or J_{ATP} could be detected between HC and KC I nominally Ca^{2+}-free solution, force development and metabolic rates in HC did not differ from those in Ca^{2+}-containing solution. Glucose emoval abolished lactate formation in HC. The temporal variations of J_{O_2} and active force during 30 min of HC and KC ere followed In HC force development increased with time whereas in KC it decreased Tension-independent J_{O_2} declined with time in HC but stayed constant in KC On the other hand tension-dependent J was unchanged in HC but declined in KC. The results of this study indicate that despite alterations in overall cell metabolism caused by the hypertonic solution the energetic aspect of cross-bridge operation may be similar in HC and KC, despite prominent differences in dynamic mechanical properties

Key ord Smooth muscle hyperosmolality contraction, metabolic rate oxygen, lactate

s ell known that hypertonic media influence the ntractile and metabolic properties of muscle In eletal muscle an increased st ffnes of the elastic ement is seen (Jewell & W lkie 1958) as well as a duced speed of active shortening (Howarth 1958). addition contracture appears in the nonstimulated muscle on e posure to hypertonic medium ännergren & Noth 1973) In smooth muscle of the t portal vein similar mechanical effect were de ribed by Andersson et al. (1974) and ha e been rther characterized in the concomitant paper Hellstrand & Arner 1980) Metabolic responses in yperosmotic media have been in estigated in keletal muscle by measurement of oxygen consumption and lactate formation (Muller 1962, Clausen 1968) as well as of heat production (Yamada 1970) A consistent finding in these investigations was increased metabolic rate in hypertonic media. Howe er no correlation of metabolic rate with tension development under these conditions has been made A general relationship between metabolic rat and shortening velocity of muscles has been proposed (Rüegg 1971). A greater rate of ATP turnover would thus correspond to a greater shortening velocity and a greater energetic cost of tension maintenance The possibility arises that such a relation might exist also in different contractile states of an indi idual muscle Suggestion has

Table 1. Mean active tension ($\hat{P}$), O_2 consumption (J_{O_2}), lactate production (J_{LA}) and calculated ATP turnover (J_{ATP} = 6.42 × J_{O_2} + 1.25 × J_{LA}) (cf. Peterson & Paul 1974) at long (L) and short (S) muscle length

N-tris = normal tris-buffered medium. K-tris = K-high (100 mM) isosmotic solution. Ca^{2+}-concentrations as indicated. Each portal vein was exposed to, in sequence, n-tris (2.5 mM Ca^{2+}), n-tris (0 mM Ca^{2+}) and either K-tris or hyperosmotic solution. n-tris data collected from both groups, but not including such data from muscles used with NaCl-hyperosmotic solution. *** = significant (p<0.05, 0.01, 0.001 resp.) difference between results at long and short muscle length as obtained by Student's t-test for paired data.

Solution	$[Ca^{2+}]$ (mM)	Muscle length	$\hat{P}$ (mN/mm²)	J_{O_2} (µmol/min g)	J_{LA} (µmol/min g)	J_{ATP} (µmol/min g)	n
	2.5	L	2.5±0.4	0.57±0.05	0.22±0.05	3.9±0.3	12
		S	0	0.41±0.03 **	0.13±0.04	2.9±0.2	
	0	L	0	0.39±0.02	0.13±0.02	2.6±0.2	12
		S	0	0.38+0.03	0.14±0.03	2.6±0.2	
K-tris	0.5	L	6.3±1.1	0.48±0.06	0.29±0.08	3.4±0.4	6
		S	0	0.43+0.05	0.15±0.03	2.9±0.3	
300 mOsm sucrose	2.5	L	6.2±0.3	0.46+0.03	0.69±0.08	3.8±0.3	6
		S	0	0.39±0.03	0.61+0.06	3.2±0.3	
300 mOsm NaCl	2.5	L	7.2±0.8	0.51±0.01	0.65±0.04	4.1±0.1	4
		S	0	0.34±0.01	0.59±0.02	2.9±0.1	

ocular scale. The muscle was then removed, blotted and weighed on a Cahn electrobalance. Metabolic data are expressed relative to the tissue wet weight. Force values at lengths are normalized to cross sectional area, which was calculated from optimal length and wet weight, assuming uniform thickness and a density of 1.05 mg/mm³.

RESULTS

Table 1 shows measured mean active tension ($\hat{P}$), oxygen consumption (J_{O_2}), lactate production (J_{LA}) and calculated ATP turnover (J_{ATP}, cf. Peterson & Paul 1974) in portal veins. The muscles, at two different lengths, were either kept in normal solution with or without Ca^{2+} or contracted by K or hyperosmolality. In normal solution the greater active tension development at long length (L), measured as mean tension ($\hat{P}$) during spontaneous activity, was accompanied by increased J_{O_2} and J_{ATP}, whereas J_{LA} in this study was not *different* from that at short length (S). In normal solution without Ca^{2+}, where the muscle was relaxed, no differences in metabolic fluxes were seen between the two lengths. In K-high solution (0.5 mM Ca^{2+}), where a contracture (KC) develops, J_{O_2}, J_{LA} and J_{ATP} were greater at the long length, but when total rates are compared as in Table 1, this difference is significant only in J_{ATP}. Hyperosmolality contractures (HC) were produced by two different agents, sucrose and NaCl. Tension development in both media were about the same, and similar to that in KC. In HC, all metabolic fluxes increased with tension, although in the total rates this difference was significant in J_{O_2} and J_{ATP} only. J_{LA} values were much increased compared to the rates measured in isotonic solutions. This increase was similar for both agents used in producing hypertonicity.

A better resolution of the tension-dependent rates of metabolism was desired than that apparent from the total rates in *Table 1*. The J_{O_2}, J_{LA} and J_{ATP} measured in Ca^{2+} free solution were subtracted from those in the activated muscle at corresponding length. The resulting quantities, called here ΔJ_{O_2}, ΔJ_{LA} and ΔJ_{ATP}, are shown plotted against mean active tension $\hat{P}$ in Fig. 1. HC data for sucrose only are shown since NaCl data were similar. The increase in ΔJ_{O_2} from short to long muscle length is significant for all modes of activation. The $\Delta J_{O_2}/\hat{P}$ slope is much steeper in normal tris than in HC and KC. At short length ΔJ_{O_2} in HC is smaller than in KC and, in fact, it is negative. At the long length, ΔJ_{O_2} in HC is lower than in KC, but the $\Delta J_{ATP}/\hat{P}$ slope is about the same. The much greater ΔJ_{LA} in HC compensates for the difference in ΔJ_{O_2}, so that ΔJ_{ATP} values, both at long and short muscle length, are about the same in HC and KC. It is notable that the tension dependence of ΔJ_{LA} for all modes of activation is weak, since there were no significant differences in ΔJ_{LA} between the muscle lengths.

The increased J_{LA} in the hyperosmotic medium is a prominent finding, and it was considered interest-

been made of such an effect in the portal vein when phasic and "tonic" activation is compared (Hellstrand 1977 1979) It would thus be of interest to know whether the low shortening speed in hyper tonic medium has a counterpart in a low rate of energy turnover

In the present investigation we have measured oxygen consumption and lactate production of the rat portal vein during exposure to hyperosmotic solution (about twice normal osmolality) under simultaneous recording of ismetric tension A com parison was made of the energetic aspects of the contractures developed in this medium and in K high isotonic medium In addition a comparison with the energitics of spontaneous phasic activity in normal medium was obtained The results indicate that whereas the lactate production is greatly enhanced in the hyperosmotic solution oxygen consumption is slightly reduced so that the overall ATP-production seems to be about the same as in the K -contracture at normal tonicity It was not possible to show any difference in the cost of con traction between the contractures in hypertonicity and isotonicity

METHODS

Portal veins from male Sprague-Dawley rats (200–400 g) were dissected and mounted in a glass chamber equipped with an O_2 electrode and a stirrer as described by Hellstrand (1977) In the present series of expts the perspex plug sealing the chamber was replaced by one made of glass of similar shape Furthermore the muscle was now fixed to the tension transducer via a fine glass capillary instead of a silk thread No difference in perfor mance of the apparatus was noted as a result of these modifications of the technique The chamber could be perfused by solution or sealed for measurement of O_2 consumption In the present expts the perfu ion rate was about 0 3 ml/min S nce the chamber volume was 1.3 ml this means that at constant rate of lactate production steady state lactate concentration was achieved in about 15 min (see Hellstrand 1977) Samples of the perfusate were collected and assayed for lactate by the fluorimetric method of Lowry & Passonneau (1972) using an Aminco-Bowman spectrophotofluorometer This method is more sensitive than the previously used spectrophotometric method and allowed a higher flow rate to be used

The solutions used were a follows (1) normal tri solution of composition in mM NaCl 120 KCl 6 0 $MgCl_2$ 1.2 $CaCl_2$ .5 glucose 11.5 Na_2Ca-versenate 0 026 and tris-(hydroxymethyl)aminomethane (Trizma Base Sigma Chemical Co) 23 titrated with HCl to a pH of 7 4 at 37°C (2) K-high tris of composition as normal tris ex cept that 100 mM of NaCl had been replaced by KCl and that the concentration of $CaCl_2$ was 0.5 mM (3) nominally Ca^{2+}-free tris obtained by omitting $CaCl_2$ in n mal tris (4) hyperosmotic solutions obtained by sucrose mannitol or NaCl to the normal or n Ca^{2+} free tris Osmolality was routinely checked Advanced Instruments Inc freezing point osm (31–LAS) and was about 290 mosm/kg in the nor 590 mosm/kg in the hypertonic media All solutions bubbled with air prior to entry into the chamber temperature of the system was kept at 37°C To bacterial contamination penicillin G (100 mg/l) and tomycin (300 mg/l) were added to the solution Na-salts After the muscle had been mounted chamber at a preload of about 5 mN it was left commodate in normal tris under perfusion for 60 m

A conditioning 30 min exposure to the hyper medium with 2.5 mM Ca was made resulting slowly developing contracture (HC). Thereafter the tocol was as follows. (1) normal tris for 45 min, (2) nally Ca free tris for 30 min (3) hyperosmotic (2.5 mM Ca^{++}) for 30 min This protocol was follo two different lengths of the muscle one longer at a tension of about 5 mN (optimal for tension develo and one shorter where the muscle developed tension. In half of the expts the muscle was kept longer length first and then shortened, wherea other half the sequence was reversed To ensure exchange of solution flow was kept higher for the initial 5 min in each solution The metabolic urements of lactate production (J_{LA}) and O_2 consum (J_{O_2}) were made during the final 15 min in each so During this period the perfusate was first collected min for lactate assay Then the flow was stopp during the final 10 min the chamber was kept closed measurement (see Hellstrand 1977) Isometric measured during this period is reported. Blanks taken from the inflow solution at times correspo the lactate samples. The samples and blanks were frozen until analysis In expts involving K-contr (KC) the above protocol was followed, except th high tris replaced the hyperosmotic solution A m tion of the protocol where the period in normal tr above) was omitted was followed for a series of where HC was induced by NaCl instead of by suc

The influences of Ca ions and glucose were ex in expts where HC was induced by the addition nitol In these expts the muscles were kept at length throughout

In a separate study the variations of J_{O_2} during 30 min periods of KC or HC was followed series of expts contractures (KC or HC) were ind two different muscle length with preceding dete tions of J in Ca -free solution The chamber wa closed and isometric tension and J_{O_2} were recorded ords were also stored on magnetic tape (Racal the Store 4 instrumentation recorder) for later co analysis (Alpha-LSI minicomputer) Data from the min in each contracture (including solution exc were discarded and the rest of the period was divid 5 intervals of 5 min each Mean tension and J interval were calculated by the computer

At the end of each expt the muscle was returned optimal length which was measured by microscop

1 Mea acth tension ($\hat{P}$) O consumption (J) lactate production (J) and c lculated ATP er J $_{TP}$=6 42×J_{O_2}+1.25×J_{LA} (cf Peterson & Paul 1974) at long (L) and short (S) m scle l ngth

= normal tris-buffered medium, K -tris = K -high (100 mM) isosmotic solution. Ca^{2+}-concentrations as indicated portal vein was exposed to, in sequence, n-tris (2 5 mM Ca^{2+}), n-tris (0 mM Ca^{2+}) and either K -tris or hyper tic solution. n-tri data collected from both groups, but not including such data from muscles used with NaCl-osmotic solution. *** = significant (p<0 05 0 01 0 001 resp.) difference between results at long and short le length as obtained by Student's t-test for paired data

ion	[Ca^{2+}] (mM)	Muscle length	$\hat{P}$ (mN/mm²)	J_{O_2} (µmol/min g)	J_{LA} (µmol/min g)	J $_{TP}$ (µmol/min g)	
	2.5	L	2.5±0 4	0 57_0.05	0 22±0 05	3 9±0 3	12
		S	0	0 41±0 03 **	0_3±0 04	2.9±0_	
	0	L	0	0.39±0 02	0.13±0 02	2.6±0..	12
		S	0	0.38±0 03	0.14±0 03	2 6 0	
ris	0.5	L	6 3 1 1	0 48+0 06	0 29±0 08	3 4±0 4	6
		S	0	0 43±0 05	0 15±0 03	2 9±0 3	
300 mOsm rose	5	L	6_±0 3	0.46±0 03	0 69±0 08	3 8±0.3	6
		S	0	0 39±0 03	0 61±0.06	3 2±0 3	
300 mOsm Cl	2 5	L	7.2±0 8	0 51±0 01	0 65 0 04	4 1±0 1	4
		S	0	0 34±0 01***	0 59±0 02	2.9±0.1	

ocular scale The muscle was then removed, blotted weighed on Cahn electrobalance. Metabolic data are ressed relati to the tissue wet eight Force values at lengths are normalized to cross sectional area. Each s calculated from optimal length and wet weight, as-ning uniform thickness and density of 1.05 mg/mm

.SULTS

able 1 shows measured mean active tension ($\hat{P}$), ygen consumption (J) lactate production (J_{LA}) d calculated ATP turnover (J $_{TP}$, cf Peterson & ul 1974) in portal veins. The muscles, at two fferent lengths were either kept in normal sol-ion, with or without Ca^{2+} or contracted by K or yperosmolality In normal solution the greater ac e tension development at long length (L) meas-ed as mean tension ($\hat{P}$) during spontaneous activi- was accompanied by increased J_{O_2} and J $_{TP}$, hereas J_{LA} in this study was not different from that short length (S) In normal solution without Ca^{2+} here the muscle was relaxed, no differences in etabolic fluxes were seen between the two engths. In K -high solution (0.5 mM Ca^{2+}) where a contracture (KC) develops, J_{O_2}, J_{LA} and J $_{TP}$ were reater at the long length, but when total rates are ompared, as in Table 1 this difference is signif cant only in J $_{TP}$. Hyperosmolality contractures HC) were produced by two different agents su-crose and NaCL Tension development in both media ere about the same and similar to that in KC In HC all metabolic fluxes increased with tension, although in the total rates this difference was significant in J_O and J $_{TP}$ only J_{LA} values were much increased compared to the rates measured in isotonic solutions This increase was similar for both agents used in producing hypertonicity

A better resolution of the tension-dependent rates of metabolism was desired than that apparent from the total rates in Table 1 The J_{O_2}, J_{LA} and J $_{TP}$ measured in Ca^{2+}-free solution were subtracted from those in the activated muscle at corresponding length. The resulting quantities, called here ΔJ_{O_2}, ΔJ_{LA} and ΔJ_{TP} are shown plotted against mean active tension $\hat{P}$ in Fig. 1 HC data for sucrose only are shown, since NaCl data were similar The increase in ΔJ_{O_2} from short to long muscle length is significant for all modes of activation The ΔJ /$\hat{P}$ slope is much steeper in normal tris than in HC and KC. At short length ΔJ in HC is smaller than in KC and in fact it is negative At the long length, ΔJ_{O_2} in HC is lower than in KC but the $\Delta J_{O_2}/\hat{P}$ slope is about the same The much greater ΔJ_{LA} in HC compensates for the difference in ΔJ_O so that ΔJ_{TP} values both at long and short muscle length, are about the same in HC and KC It is notable that the tension dependence of ΔJ_{LA} for all modes of activation is weak since there were no significant differences in ΔJ_{LA} between the muscle lengths

The increased J in the hyperosmotic medium is prominent finding, and it was considered interest

Table 2. *Isometric tension and metabolic fluxes in isosmotic and hyperosmotic solutions with and without glucose*

Significance levels as in Table 1. In each group, 2 muscles were exposed first to glucose-free and then to glucose-containing media, whereas the 2 other muscles were treated in the reverse order

Solution	[Ca^{2+}] (mM)	[glucose] (mM)	$\hat{P}$ (mN/mm²)	J (µmol/min × g)	J_{La} (µmol/min × g)	
n-tris	0	11.5	0	0.27±0.02	0.1 ±0.03	4
	0	0	0	0.30±0.04	0.02±0.02*	4
n-tris+300 mOsm	2.5	11.5	6.8±1.0	0.32±0.02	0.49±0.08	4
mannitol	2.5	0	8.3±1.5	0.27±0.01	0.02±0.03*	4

ing to see what effect omission of glucose in the medium would have in this situation. In Table 2 are summarized 4 expt where HC is compared to the relaxed (Ca^{2+} free) state in the presence and absence of glucose. To avoid possible glucose contamination HC was produced by mannitol rather than by sucrose. Tension development in HC was not dependent on presence of glucose. J_L was essentially zero in glucose-free solution both in the relaxed and contracted muscle. A slight, non-significant increase in J in glucose-free solution was seen in both mechanical states.

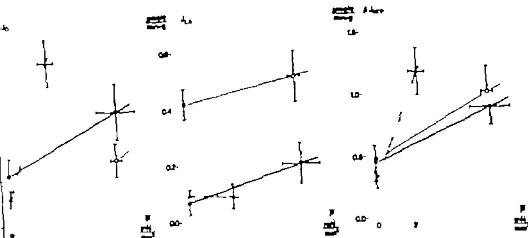
The effects of nominally Ca^{2+} free solution on tension and metabolic fluxes in HC are shown in Table 3. *Tension development in HC was not dependent* on external Ca^{2+} as already observed (Andersson et al. 1974). In HC J and J_L were unchanged whether Ca^{2+} was present or not.

The results presented so far were all obtained from measurements made late during long-standing contractures (30 min). It was considered possible that the metabolic flux rates might be different earlier in the contractures. With the technique used the time resolution of the J_L measurements was not adequate to bear on this question. J however can be followed with accuracy during the course of the contraction (see Methods). Fig. 2 shows data on the temporal variations in mean tension ($\hat{P}$) and J during 30 min contractures, divided into five 5 min intervals. Time zero is taken to be about 5 min after the initiation of contraction by solution change. ... expts were done on muscles giving KC and HC responses. The left panel shows force at long and short muscle lengths (open and closed symbols). Circles indicate KC and squares HC. The difference in length was the same in both kinds of ... (short=40% of long, approx.), but whereas there was no tension development at short length in KC some tension was seen in HC. HC continued to increase during the exposure period whereas KC declines from an initial high tension. The J values are shown in the middle panel. In KC at long length J_{x_2} declines with time whereas at short length it is constant. In HC however J declines with time both at long and short length despite the increase in tension. The tension dependence of J was evaluated by computing the quotient $(J^L - J^S)/(\hat{P}^L - \hat{P}^S)$ where the superscripts L and S mark muscle length. This is shown in the right panel. It is seen that the quotient remains at the same value throughout the contraction in HC whereas in KC it declines with time. At the end of contraction the quotient is seen to be smaller in KC than in HC in contrast to the data shown in Fig. 1. We have no explanation for this variability but it is notable that in this series the developed tension (left panel

Table 3. *Isometric tension and metabolic fluxes in hyperosmotic Ca^{2+}-free solution and in hyperosmotic solutions with and without added Ca^{2+}*

Significance levels as in Table 1. Similar protocol as in Table

Solution	[Ca^{2+}] (mM)	[glucose] (mM)	$\hat{P}$ (mN/mm²)	J (µmol/min × g)	J (µmol/min × g)	n
n-tris		11.5	0	0.4 ±0.02	0.1±0.0	4
n-tris+300 mOsm		11	8.3±1.1	0.43±0.0	0.77±0.04	4
mannitol			8.7±1.3	0.44±0.04	0.72	4



1 Rates of O_2 consumption (ΔJ_{O_2}) lactate production (ΔJ_{LA}) and calculated ATP production ($\Delta J_{\sim P}$) above in Ca^{++}-free solution vs. mean active tension (P) Triangles, broken line: normal tris solution (2.5 mM Ca^{2+}); full line K -high solution (0.5 mM Ca^{++}) squares, dotted line, hyperosmotic solution (sucrose 2.5 mM Ca^{2+}). d symbols short length, open symbols long length Data shown as mean values ±S E.

smaller in KC The results of these expts thus cate that in HC the tension-independent J ines with time whereas in KC it is constant. On other hand, in KC there is a decrease with time he tension-dependent J_{O_2}.

DISCUSSION

starting point for this investigation was the umption that the greatly reduced shortening ed of the portal vein in hyperosmotic solution dersson et al 1974 Hellstrand & Arner 1980) ild have its counterpart in a reduced metabolic t of tension development We chose to compare hyperosmolality contracture (HC) with K uced contractures (KC) of similar tension, and with the normal spontaneous activity of the both muscle quantitated as integrated (mean) sion. Values of J_{O_2} and J in spontaneous activi- and J_{O_2} in K -contractures of the portal vein were orted by Hellstrand (1977) In the present work a re sensitive lactate assay was employed which wed for higher flow rates through the chamber l thus a better time resolution This enabled us to asure J_{LA} in sustained contractures as well as in ady state pontaneous activity Generally J_{LA} ues found here were lower than those reported lier and in spontaneous activity no tension dependence of J_{LA} was found (Fig 1 middle panel) In trast to the earlier study

Both in KC and HC greater J_{O_2} was found when isometric tension was greater (Fig 1 left panel) This tension dependence of J_{O_2} was similar in HC and KC and much smaller than in spontaneous activity J_{LA} was at least twice as great in HC as in KC (Table 1) When the total ATP-turnover was calculated according to standard stoichiometry (cf Peterson & Paul 1974) similar values were found in KC and HC, both in absolute amounts and in relation to developed tension (Table 1 and Fig. 1 right panel)

Increased lactate production in hypertonic medium has already been observed in skeletal muscle (Clausen 1968) Possible causes of this effect could be stimulation of glycolysis as a result of increased glucose uptake accelerated glycogenolysis (Clausen 1968) inhibition of Krebs cycle or respiratory uncoupling. Hyperosmolality might be able to stimulate some mechanism preferentially utilizing glycolytically supplied energy A linkage between ionic pump activity and glycolysis has been suggested (Muller 196., Pease Bauer & Paul 1979)

For the interpretation of these results it should be kept in mind that most of our metabolic measurements were performed late in long-standing contractures, whereas in the parallel mechanical expts (Hellstrand & Arner 1980) the K -contractures for technical reasons were kept at only a few min duration. It is possible that force velocity measurements performed later during a K -contracture would

Table 2 *Isometric tension and metabolic fluxes in isosmotic and hyperosmotic solutions with and without glucose*

Significance levels as in Table 1 In each group 2 muscles were exposed first to glucose free and then to glucose containing media whereas the other muscles were treated in the reverse order

Solution	[Ca] (mM)	[glucose] (mM)	P (mN/mm²)	J (μmol/min×g)	J (μmol/min×g)	
n-tris	0	11.5	0	0 ?7±0 0	0 17±0 03	4
	0	0	0	0 30±0 04	0 02±0 02*	4
n-tris+300 mOsm mannitol	5	11 5	6 8±1 0	0.3 ±0 0?	0 49±0 05	4
	?.5	0	8 3±1.5	0 37±0 01	0 0 ±0 01	4

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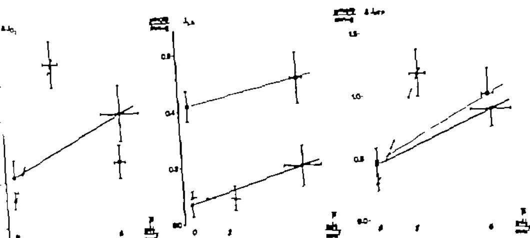
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Table 3 *Isometric tension and metabolic fluxes in isosmotic Ca² free solution and in hyperosmotic solutions with and without added Ca*

Significance levels as in Table 1 Similar protocol as in Table 2

Solution	[Ca] (mM)	[glucose] (mM)	P (mN/mm)	J (μmol/min g)	J (μmol/min×g)
n-tris	0	11 5	0	0 42±0 02	0_1±0 02
n-tris+300 mOsm mannitol	5	11 5	8.3±1 1	0 43±0 02	0 77±0 04
	0	11 5	8 7±1.3	0 44±0 04	0 7?±0 04



1 Rates of O_2 consumption (ΔJ_{O_2}), lactate production (ΔJ_{LA}) and calculated ATP production ($\Delta J_{\sim P}$) above in Ca^{2+}-free solution vs. mean active tension ($\bar{P}$). Triangles, broken line: normal tris solution (2.5 mM Ca^{2+}) full line: K-high solution (0.5 mM Ca^{2+}), squares, dotted line: hyperosmotic solution (sucrose 2.5 mM Ca^{2+}). ... symbol: short length, open symbols: long length. Data shown as mean values ±S.E.

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...CUSSION

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Increased lactate production in hypertonic medium has already been observed in skeletal muscle (Clausen 1968). Possible causes of this effect could be stimulation of glycolysis as a result of increased glucose uptake accelerated glycogenolysis (Clausen 1968) inhibition of Krebs cycle or respiratory uncoupling. Hyperosmolality might be able to stimulate some mechanism preferentially utilizing glycolytically supplied energy. A linkage between ionic pump activity and glycolysis has been suggested (Muller 1962, Pease Bauer & Paul 1979)

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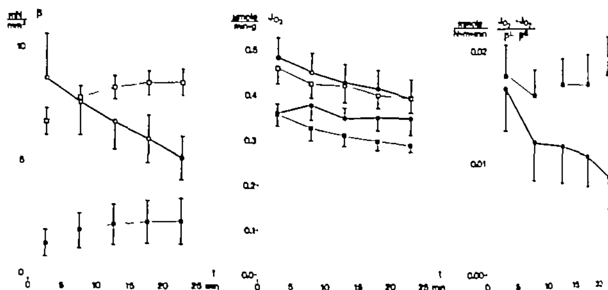


Fig 2 Variation in developed tension ($\hat{P}$) O_2 consumption (J_{O_2}) and tension-related O_2 consumption [($J_{O_2}^L$ − $J_{O_2}^S$)/($\hat{P}^L$ − $\hat{P}^S$)] during the course of 30 min contractures at short (S) and long (L) muscle lengths. Circles, full K+ high solution (0.5 mM Ca²+) squares dotted line hyperosmotic solution (sucrose ..5 mM Ca²+). Full ... short length open symbols, long length Data shown as mean values ±S E, 6 expts in each group

have given a lower maximal shortening velocity Results indicating such an effect in amphibian slow twitch fibres have been reported by Lännergren (1978) However the difference in shortening velocity between KC and HC of the portal vein is so great (about 5-fold) that it appears unlikely to be entirely abolished even when measurements are made late in contractures of both kinds It is to be noted that the tension-related J_O seems to diminish with time in KC in contrast to HC (Fig 2 right panel)

The tension development in HC occurs independent of membrane potential and at levels of extracellular [Ca²+] unable to sustain a KC (Andersson et al 1974 McGrath & Shepherd 1976 Hellstrand & Arner 1980) Work on skeletal muscle suggests that hypertonic solutions may mobilize Ca²+ ions from intracellular stores and also increase membrane permeability for Ca (Yamada 1970 Lännergren & Noth 1973 Homsher Briggs & Wise 1974 Clausen Dahl Hansen & Elbrink 1979) It is also notable that Ca accumulation by isolated smooth muscle mitochondria is inhibited in hyperosmotic solution (S Batra personal communication) Thus it is possible that at least part of the tension increase in the portal vein in hyperosmotic solution is due to activation of the contractile proteins by elevated [Ca]. At lower degrees of hyperosmolarity (below 100 mosm/l) an increase is seen in peak force of portal vein spontaneous contractions (McKinley McKenzie & Blair West ... and K -contractures (S Sigurdsson personal communication)

The maximal tension development of a HC ... portal vein is less than that of a KC in activation at optimal Ca²+-concentration. Expts on ... minated skeletal muscle reveal decreased tension development at increased ionic strength (Gordon ... 1973 Homsher et al 1974) At high ionic strength actomyosin ATPase of vascular smooth muscle ... decreased (Maxwell Bohr & Murphy 1971) ... depression of the maximal contractile activity ... the changes in stiffness and shortening ... caused by hypertonic solutions seem to be ... separate from the contractile activation and the apparent increase of cytoplasmatic Ca It is not ... whether HC is entirely caused by Ca²+-... mechanisms (increased Ca level and/or more ... Ca sensitivity of the contractile system) ... after prolonged depletion of Ca²+ about 50 % of ... control HC response can be elicited (Andersson ... al 1974) The possibility of activation independent of Ca is considered in the concomitant ... (Hellstrand & Arner 1980)

The results of this study do thus not support ... hypothesis that the low maximal shortening ... ty of the contracture in the hypertonic medium ... have a counterpart in a lower metabolic co... tension Rather the results seem to be compa... with a situation in the hypertonic medium wh...

...ed number of cross-bridges operate in an ...tially normal way. The possibilities of rigor ...nkages and/or ultrastructural alterations in the ...actile apparatus in hypertonicity have been ...ssed (Hellstrand & Arner 1980). Such factors ...t reduce the number of active cross-bridges and ...uce an internal resistance to shortening.

...ould like to thank Monica Lundahl, Monica ...mboim and Ian Nordstrom for technical assistance. ...nvestigation was supported by grants from the ...lish Medical Research Council (04X-0028), the ...cal Faculty, University of Lund and AB Hässle, ...borg.

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ex inhibition of sympathetic activity during volume l in awake normotensive and spontaneously ertensive rats

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RICKSTEN S.-E. & THOREN P Refle inhibition of sympathetic activity during olume load in awake normotensiv and spontaneously hypertensive rats. Acta Physiol Scand 1980. 110 77–82. Received 17 Dec 1979 ISSN 0001-6772. Department of Physiology University of Goteborg S eden.

The refle inhibition of the sympathetic activity in the splanchnic nerves was recorded upon volume expansion with blood in awake spontaneously hypertensive rats (SHR) and in normotensive Wistar Kyoto rats (WKR) at an age of 16–20 weeks. At 10% blood volume expansion SHR showed significantly greater nerv inhibition (43%) in comparison with WKR (33%). This augmented reflex response was not caused by the arterial baroreceptors, because the sensitivity of the arterial baroreceptor reflex arch if anything, tended to be lower in SHR and the increase in arterial blood pressure upon volume load was also lower in SHR. It is suggested that the reason for this increased reflex inhibition in SHR is an augmented low pressure receptor response. The mechanism behind this is discussed. The most likely explanation is decreased distensibility of the venous system the systemic and/or the pulmonary veins

Key ord A ake rats, sympathetic nerve recording SHR, WKR, volume load

intrathoracic low pressure mechanoreceptors of great importance for the reflexogenic control he cardiovascular system. There are two types eceptor endings in the cardiopulmonary region h ith afferent travelling in the agal nerves type of eceptor-endings is mainly located at junction of the great eins with the right and left ia. These atrial receptors have rapidly conduct medullated afferent Upon activation these re ptors evoke reflex tachycardia and water diuresis inden 1976, Thoren 1979) The large population receptor-endings with nonmedullated agal af rent are located both in the atria, pulmonary ins and in the left entricle (Thorén 1979) An tivation of these receptors induces reflex adycardia and inhibition of sympathetic discharge specially to the kidney

Thoren et al (1979) wer the first t study these ceptors in genetic hypertension. Using the spon- aneously hypertensiv rat (SHR) they found that e cardiopulmonary receptors with nonmedullated fferent er clearly reset A higher left atrial pressure (~10 mmHg) was needed to activate the receptors in SHR compared to normotensive control rats (~5 mmHg) Ricksten et al (1979) also studied the reflex inhibition of renal sympathetic nerve activity from the cardiopulmonary receptors in SHR. In anesthetized thoracotomized and baroreceptor denervated rats it was shown that dur ing olume load with plasma given intravenously the nerve activity started to be inhibited at a left atrial pressure around 10 mmHg in SHR in comparison with 5 mmHg in normotensive control rats Interestingly enough however a smaller amount of plasma was needed in SHR to reach the 'threshold left atrial pressure for reflex inhibition of the nerv activity compared to normotensive control rats In other word the low pressure receptors with nonmedullated afferents (mainly located in the left atrium in the rat, Thorén et al 1979) seem to be activated more for given increase of the plasma volume in SHR compared to normotensive control rats, but a higher pressure was also needed. As, however the animal in the mentioned study were

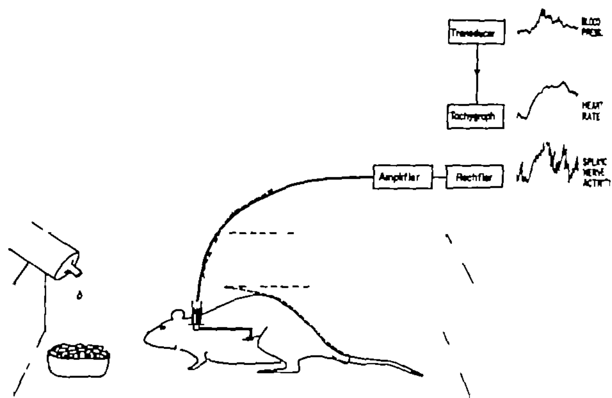


Fig 1 Schematic drawing of the preparation The experiments were performed with the animals in a cage to which they had been habituated for 36 h before the experiments and where they were provided with food and water

thoracotomized and the surgical trauma was rather extensive we wanted to examine the reflex nerve inhibition upon volume load in intact awake normotensive and hypertensive animals

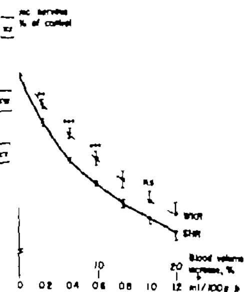
METHODS

Nine spontaneously hypertensive rats (SHR) weighing 335±12 g were used in this study 8 normotensive rats of the Wistar Kyoto (WKR) strain were used as controls, weighing 342±12 g Both groups were 4–5 months old Intraarterial blood pressure was measured in the caudal artery after light ether anesthesia some days before the surgical procedure Mean arterial pressures in SHR and WKR were 155±4 mmHg and 107±1 mmHg, respectively During Nembutal® anesthesia the coeliac ganglion and the splanchnic nerves were identified via a left retroperitoneal incision. A thin bipolar silver electrode was placed around a branch of the splanchnic nerve and isolated carefully with silicone rubber (Wacker Sil Gel 604). The animals were then allowed to recover for at least 36 h Splanchnic nerve activity was recorded via a cable connected to an adaptor on the back of the animal (Fig 1) The nerve signal was amplified (Grass p 511) and rectified and the mean nerve activity was displayed on a Grass Polygraph (model 7). Arterial pressure and heart rate were continuously measured via a catheter placed in the caudal artery (see Fig. 1) The animals were placed in their own cages and received food and water

In 11 of 17 expts. it was necessary to sedate the animals with diazepam 50 µg i.v (Valium®) in order to get a stable control level of sympathetic nerve discharge Though this dose of Valium sedated the animals they could easily be awaked and aroused by e g touch stimuli. The administration of Valium did not change the basal level of sympathetic nerve discharge but decreased the emotionally elicited sympathetic excitations otherwise often seen in these awake animals.

EXPERIMENTAL PROCEDURES

Initially baroreceptor sensitivity was estimated by an i.a noradrenaline infusion which raised mean arterial pressure around 50 mmHg over ~3 min The pressure change was plotted against baroreceptor-induced inhibition and baroreceptor sensitivity was defined as percentage nerve inhibition per mmHg of arterial pressure rise After a control period of about 30 min the animals were then volume loaded i.a. with blood from a donor of the same colony Splanchnic nerve activity expressed as per cent of control activity was then plotted against degree of blood volume expansion The volume load was expressed as ml/100 g b w and also as per cent blood volume increase Rippe et al (1978) have shown that the blood volume is about 6 ml/100 g in both SHR and normotensive control rats. Addition of 0 6 ml/100 g of blood therefore corresponds to a blood volume expansion of about 10% in both SHR and W... each experiment ... postmortal nerve activity ... for



Splanchnic nerve inhibition during volume load blood in awake SHR and WKR. The change in blood ... is expressed in absolute terms and in percentage ... (*P<0.05 **P<0.01 ***P<0.001).

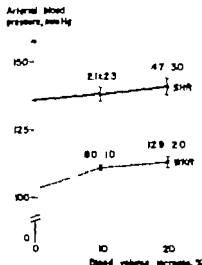


Fig. 3. The change in mean arterial pressure upon volume load in SHR and WKR. The repeated injections of small blood amounts (0.5 ml) were made intraarterially. Note that SHR increased arterial pressure less than WKR for a certain change in blood volume.

...ve the noise activity which was 0.8–0.9 μV. Group ...parison *t*-test was used for the statistical evaluations. ... value <0.05 was considered as statistically signifi...

...SULTS

... estimated baroreceptor sensitivity was not ...tically different between the WKR and the ...R, although it tended to be reduced in SHR, ...±0.2%/mmHg compared to 2.0±0.3 in WKR.

...ig. 2 shows the mean values of sympathetic ...e inhibition plotted against degree of blood vol... expansion. At 10% blood volume load SHR ...wed a significantly greater nerve inhibition ...%) compared with WKR (33%) while the differ...ce was not significant at more extensive volume ...pansion. This difference in response between ...IR and WKR was not due to an augmented inhibi...n from the arterial baroreceptors in SHR, be...use arterial pressure increased indeed less in ...IR than in WKR (*Fig. 3*) upon blood volume load.

From the data on baroreceptor sensitivity and ...anges in arterial pressure the relative contribu...n of arterial baroreceptors to sympathetic nerve ...hibition upon volume load could be estimated ...ig. 4). From the figure it is clear that the relative contribution of arterial baroreceptors is less in SHR, despite the fact the the degree of sympathetic inhibition is greater in SHR upon 10% volume load. It is likely that cardiopulmonary receptors are responsible for the remaining (non-arterial baroreceptor dependent) inhibition (Ricksten et al. 1979, Thorén 1979).

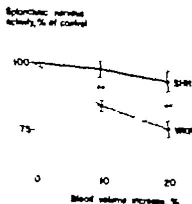


Fig. 4. The estimated contribution of arterial baroreceptors to reflex sympathetic inhibition in response to volume load. The data are obtained from the value of arterial baroreceptor sensitivity (see text) and changes in mean arterial pressure upon volume load (Fig. 3).

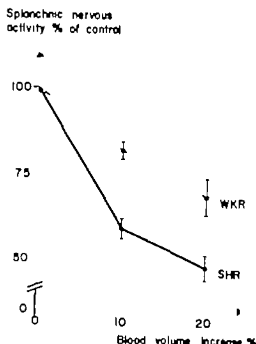


Fig 5 The estimated contribution of cardiac receptors to reflex sympathetic inhibition in response to volume load The curve is derived from a subtraction of the values in Fig 4 from the corresponding values in Fig 2

Fig 5 shows the estimated reflex sympathetic inhibition evoked from cardiopulmonary receptors This figure is derived from the difference between total sympathetic inhibition (Fig 2) and that caused by arterial baroreceptors (Fig 4) Fig. 5 shows that a 10% and ?0 % volume load activates cardiopulmonary receptors more in SHR leading to an exaggerated reflex inhibition of splanchnic nerve activity in hypertensive animals compared to normotensive ones

DISCUSSION

The most significant finding in this study is the fact that SHR shows a more pronounced reflex inhibition (Fig. ?) of splanchnic sympathetic activity upon volume load than normotensive controls Thus at a 10% increase of blood volume sympathetic discharge decreased significantly more in SHR than in WKR We here assume that the total blood volume per 100 g b w is about the same in SHR and WKR as reported by Rippe et al (1978) Other investigators report a 1? 5% greater blood volume in SHR compared to normotensive Wistar rats (Nikodyevic et al 197?) If this is true the reflex inhibitory response to a given volume load (ml/100 g) would be even more marked than shown in Fig. ? In a recent study it is shown that the total volume is 10% greater in WKR compared to S an age of 3–4 months (Lundin personal communication) If Fig ? is recalculated accord Lundin s data there is still a significantly reflex sympathetic inhibition in SHR than at a volume expansion of 10% ($P<0\,01$).

Arterial baroreceptor contribution What is the reason for this more powerful reflex inh response to volume load in SHR? It is not med via arterial baroreceptors because if anything sensitivity of the baroreceptor reflex arch wa er in SHR Thus for a given change in a pressure SHR tended to inhibit sympathetic charge less than WKR though this difference not statistically significant Such reduced sen ty of the arterial baroreceptors in SHR have reported by several previous investigators (Sa Wang 1976 Brown et al 1976)

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Physiological significance of augmented volume reflexes in SHR

A surprising finding in the present study was that mean arterial pressure increased less in SHR than in WKR upon 10% blood volume expansion. Thus, the augmented volume receptor reflex response in SHR allows these animals to better cope with increases in blood olume in at least terms of increases in arterial pressure and therefore with less of arterial baroreceptor activation.

Another interesting phenomenon in hypertensive animals and man is the exaggerated natriuretic response to saline load (Willis et al 1976). It is possible that this is at least partly due to such an accentuated reflex sympathetic inhibition of low-pressure eceptors in SHR upon volume loading, because renal sympathetic nerves are known to markedly influence both sodium excretion (DiBona 1977) and renin release (Zanchetti 1976)

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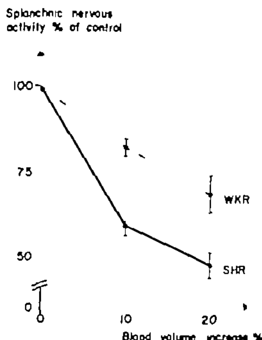


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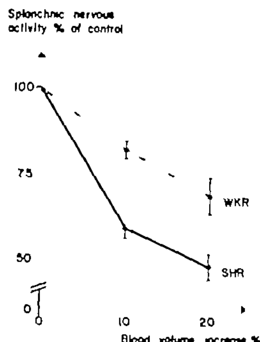


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...mporal dissociation between the negative inotropism ...d the increase in cyclic GMP level induced ...choline esters in spontaneously beating ...atria preparations[1]

...METSA KETELÄ, R. KUOSA and H. VAPAATALO
...rtment of Biomedical Sciences, University of Tampere, Finland

METSÄ-KETELÄ T., KUOSA R. & VAPAATALO H. Temporal dissociation between the negative inotropism and the increase in cyclic GMP level induced by choline esters in spontaneously beating rat atria preparations. Acta Physiol Scand 1980. 110 83–87. Received 20 Dec. 1979. ISSN 0001-6772. Department of Biomedical Sciences, University of Tampere, Finland.

The effects of acetylcholine and carbachol on cyclic GMP levels and contraction amplitude were investigated simultaneously in spontaneously beating rat atria preparations. Both drugs at the concentrations of 5 · 10⁻⁷ M or higher produced a very rapid decline of amplitude. However, the cyclic GMP level was not elevated earlier than 5 s later, when the amplitude was already reduced to 50% of the initial value. Concentrations of acetylcholine below 5 · 10⁻⁷ M were not able to affect the cyclic GMP level, although they reduced the contractility significantly. Acetylcholine had no effect on the cyclic AMP level. The data demonstrate that cyclic GMP does not mediate the negative inotropic effect of choline esters in rat atria preparations.

Key words: Acetylcholine, cyclic GMP, negative inotropism, heart

... 1970, when George et al. (1970) suggested ...possible relationship between guanosine 3',5'-...nophosphate (cyclic GMP) and the contraction ...ces of the heart, it has been concluded in several ...papers that there is a correlation between nega... ...inotropism and an increase in cyclic GMP level ... the heart (Lee et al. 1972, George et al. 1973, ...orge et al. 1975, Watanabe & Besh 1975, Fink et ... 1976) after acetylcholine (ACh) administration. ...temporal relationship between the negative ino...pism and the increase of cyclic GMP after ACh ... been reported (George et al. 1970, George et al. ...73, George et al. 1975, Fink et al. 1976) in favour ... the hypothesis on cyclic GMP-mediated negative ...otropism.

This hypothesis is contradicted by the observa...ns that low concentrations of choline esters are ... able to increase the cyclic GMP content, al...ough they reduce cardiac contractility (Diamond ... al. 1977, Brooker 1977). Some drugs, e.g. sodium ...troprusside, also increase markedly the cyclic ...MP level without any effect on contractility ...iamond et al. 1977, Katsuki et al. 1977, Keely & ...ncoln 1978). It has also been reported that ACh induces various actions on the heart including increase in cyclic GMP, but the actions are only partially mediated by cyclic GMP (Nawrath 1977).

To study the role of cyclic GMP in the negative inotropic effect of ACh we have used spontaneously beating rat atria preparations, which can be frozen very rapidly, and a 5 s timing scale in sampling during the initial period of the negative inotropism.

MATERIALS AND METHODS

Isolated spontaneously beating rat atria preparation were used. Fed male Wistar rats (weighing 190–220 g) were killed by dislocation of the neck. The hearts were immediately excised, immersed and washed free from blood in Tyrode solution containing 1000 IU/l heparin at room temperature. The Tyrode content (g/l) was: NaCl 8.0, KCl 0.2, $CaCl_2 \cdot 2H_2O$ 0.18, $MgCl_2 \cdot 6H_2O$ 0.1, $NaH_2PO_4 \cdot 2H_2O$ 0.05, $NaHCO_3$ 0.4, glucose 1.0. The pH was 7.4. The atria were separated from ventricles in heparinized Tyrode bubbled continuously with pure

[1] The results have been partly presented in VIth International Symposium on Medicinal Chemistry, Brighton 4–7 September 1978.

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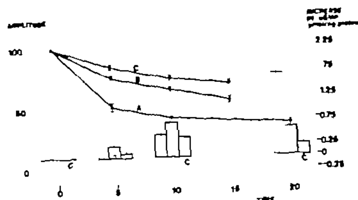


Fig. 3. Effects of various concentrations of acetylcholine on the amplitude and cyclic GMP levels of spontaneously beating rat atria preparations. The final concentrations of acetylcholine in the incubation medium were 1 10^{-6} M (A ... 8), 5 10^{-7} M (B =5) and 1 10^{-7} M (C =5). Each point represents the mean ± S.E. of the amplitude and the columns means ± S.E. of the change in cyclic GMP level. S.E. and statistical significancy (*P<0.05) has been calculated from the original values.

Tris HCl buffer pH 7.6 (Buffer I). The assay was ... ormed as follows: 50 µl of sample or standard (0.4– ... pmol/50 µl in Buffer I), 25 µl [³H]-cyclic GMP (0.5 ... in Buffer I containing 100 mM $MgSO_4$). The incubation at 0°C was initiated by adding 25 µl of cyclic BMP ... pendent protein kinase in 5 mM potassium phosphate ... fer pH 7.0, and stopped by 1 ml of 0°C potassium ... sphate buffer (20 mM, pH 6.0). One ml of this solution ... filtered through ... mixed ester cellulose filter (Millipore GSWP 0.22 µm). The disc was dried at 60–70°C for 20 min and dissolved in 500 µl of dimethylethylene... colmonoethylether. Then 4.5 ml of ... ylene based scintillator solution (Lipoluma ... Lumac AG Basel, Switzerland) ... as added and the radioactivity was measured by ... scintillation counter.

... both assays, the zero binding level was kept between ... and 25 %. The pellet of trichloroacetic acid extract was ... solved in 1 ml 1 mmol/l NaOH and the protein content ... measured by the method of Lowry et al. (1951).

... evaluate the recovery 325 µl of the resuspended ... mple ... as transferred into ... scintillation vial and 4.5 ml ... toluene based scintillator solution (Aqualuma ... Lumac ... Basel, Switzerland) was added. From the radioactivity the recovery of the extraction ... as calculated and taken ... consideration in the final concentrations. The recovery of the extraction of cyclic AMP varied from 90 to ... 0 %. The fraction used for cyclic GMP assay contained ... 75 % of [³H]-cyclic GMP added.

Chemicals. Acetylcholine chloride (Sigma Chemicals, St. Louis, Mo. USA), cyclic AMP and cyclic GMP (Boehringer Mannheim GmbH, Mannheim, Germany), [³H]-cyclic AMP and [³H]-cyclic GMP (Radiochemical Centre Ltd, Amersham, England) and the cation exchange ... AG 50W X4 (200–400 mesh, hydrogen form, Bio-Rad Laboratories, Richmond, Calif., USA).

For testing the statistical significance, Student' ... independent t-test ... as used.

RESULTS

The time courses of the effects of acetylcholine on contractility and cyclic GMP are presented in Fig. 1. The negative inotropic effect appears immediately after the administration of ACh (1×10^{-6} M) and reaches the maximum at 20 s. The initial decline of the amplitude is very rapid, and the 50 % level of the initial value is reached in 5 s. There is at least a 5 s delay in the onset of the increase in cyclic GMP level, which is elevated in 10 s (P<0.05). The maximum is reached in 20 s (P<0.01). The cyclic AMP level remains unaltered during the testing period. Carbachol (CCh), similarly to ACh, changes the contractility and cyclic GMP level (Fig. 2). Atropine (1.4×10^{-6} M) abolishes both the negative inotropic effect and the cyclic GMP elevation by ACh (1×10^{-6} M).

The dose-response curves of ACh on the increase in cyclic GMP and in amplitude are presented in Fig. 3. The threshold concentration of ACh to produce an increase in cyclic GMP level in rat atria preparation is 5×10^{-7} M. Concentrations below this are however able to depress the contractility.

DISCUSSION

Our results do not support the hypothesis suggested by George et al. (1970) that cyclic GMP could mediate the negative inotropic effect of acetylcholine. In our preparation, ACh in concentrations

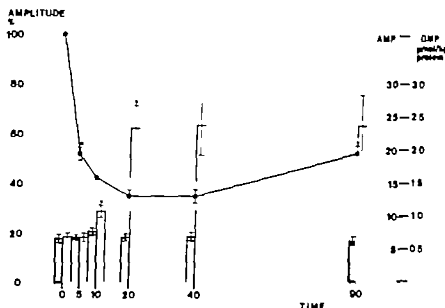
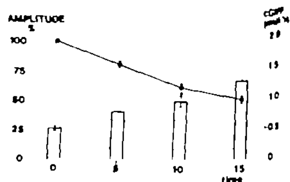


Fig 1 Effects of acetylcholine on the amplitude (●—●) on the cyclic GMP (open column) and the cyclic AMP (shaded columns) levels of spontaneously beating rat atria preparations The atria were preincubated for 45 min in Tyrode solution and then in the final concentration of 1×10 M acetylcholine Each point or column represents the mean ± S E. of 8 atria Asterisk indicates a statistically significant change ($P<0.05$) from the control value

oxygen A polyester suture was tied to the apex of both atria and they were transferred to an organ bath (?0 ml +30°C) with continuous flow (1 3 ml/min) and bubbling of pure oxygen The oxygen saturation wa monitored with a Clark s electrode (Yal Model 53 Oxygen Monitor Yellow Springs Instrument Co Yellow Springs Ohio USA) and kept at the maximum (about 95% saturation) Contrac tions of the atria were registered isometrically by Harvard Apparatus Isometric Force Transducer model 363 and Amplifier model 350 Fou preparations were stabilized at the same time after 45 min a drug wa given in a ?00 µl volume and the atria were frozen by Wollenberger clamps precooled in liqu d nitrogen The fixation procedure from cutting of suture to frozen tissue took 1 The co trac tility adenosine 3 ,5 monophosphate (cyclic AMP) and cyclic GMP were measured from the same preparat ons. The atria were stored for the analysis at − ?0°C

C lic CMP a d cycl AMP a sa Tis ues were pul erized by a m crod smembrator (B Braun Melsungen Germany) in a teflon ial precooled in liquid n troge with a 13 g steel ball The pul erized atria (100–150 mg) were put into 1.5 ml polyethylene ials (Eppendorff GmbH Eppendorff Germany) The 500 µl of 5% trichloroacetic acid wa added and the suspen ion was heated at 95°C f r 2 min The extraction of the nucleotides was promoted by vigorous shaking for 15 min at room temperature After centrifugation (9000×g 15 m n) the supernatant wa d ided into two portions 400 µl f r cyclic GMP and 50 µl for cyclic AMP a says. To the cyclic AMP al 40 µl of H_2O w added For monitoring the reco erie about 85 Bq of the corresponding tritiated cyclic nucleotide I 50 µl of H_2O was added to each sample Wat r saturated diethyl ether washing wa repe ted three times after low ring the pH .5–3 0 by 10 µl of 0 5 M HCl The cyclic AMP lyophilized and assayed without any further purific by the method of Gilman (1970)

The cyclic GMP in the 400 µl of ether washed su tant was separated from the disturbing cyclic AMP b 50W–X4 chromatography in a 9×45 mm column eluted with H_2O and a fraction from 800 to 1?00 µl collected Cyclic AMP was eluted after 1?00 µl fraction collected was lyoph lized Cyclic GMP measured by the competitive protein binding Dinnendahl (1974) using partially purified lobster muscle cy lic GMP-dependent protein kinase lyophilized cyclic GMP was resuspended in 250 µl of



F g Effect of carbacholine (5 10 M) on the amplitude (●—●) and on the cyclic GMP level (columns) Each point or column represent the mean ± S E of atria preparation ($P<0.05$)

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of 5×10^{-7} M or higher reduced at first the amplitude and a few seconds thereafter the level of cyclic GMP increased Low concentrations did not change significantly the cyclic GMP level in spite of decreased contractility

The time response curves of ACh for negative inotropic effect and for increase in cyclic GMP have been presented earlier (George et al 1970 George et al 1973 George et al 1975 Fink et al 1976) with the conclusion that cyclic GMP may mediate the negative inotropic effect of ACh The preparations used in those studies were isolated perfused rat hearts (George et al 1970 George et al 1973) and isolated paced rabbit atria (George et al 1975 Fink et al 1976) The negative inotropic response to ACh developed very slowly compared to spontaneously beating rat atria in the present study The contractility of our preparation was reduced on the same time scale as in the rabbit atria preparation in situ after stimulation of the vagus nerve (Fink et al 1976) In the paced rabbit atria preparation (George et al 1975) there seems to be no established time difference in the mechanical responses of the vagal and sympathetic nerves (Hutter & Trautwein 1956) on the heart In the preparation used in the present study the difference is more than 5 s (data not present)

Recently Keely & Lincoln (1978) have reported that both ACh and sodium nitroprusside can increase the cyclic GMP level of perfused rat heart but only ACh is able to depress the contractility From the figures of that study even the existence of a temporal difference in those two responses of ACh can be assumed

Low concentrations of ACh (5×10 M) have been reported to produce a decrease in the contractility of cat atrial strips (Diamond et al 1977) without any change in cyclic GMP This agrees with the present study in which the threshold concentration for the cyclic GMP elevation is 5×10 M CCh up to 1×10^{-4} M concentration has also been reported to be ineffective in altering the cyclic GMP level but to be able to depress very effectively the tension developed (Brooker 1977)

The cardiac contractility correlates to the oxygen consumption of the heart very well (Braunwald 1971 Theisohn et al 1977) ACh in a concentration of 1×10^{-4} M in the present study produced a very rapid decline of the contractility which could thus be expected to affect heavily the oxygen consumption and thereby the redox state The redox state and formation of free radicals have been rep[...] be the most important physiological determ[...] the activation of the guanylate cyclase (M[...] Murad 1977)

It may be possible that ACh either directly [...] reducing the contractility first disturbs the b[...] between supply and demand of oxygen and [...] the redox state which then activates the gu[...] cyclase The observation that low concentratio[...] ACh do not affect the cyclic GMP level ma[...] cate that the initial decline of contractility [...] quite severe to divert the redox-state enough fo[...] guanylate cyclase activation process.

We suggest that cyclic GMP cannot be [...] mediator of the contractility changes in ACh [...] duced negative inotropism Because the increas[...] cyclic GMP is temporally closely associated [...] but not preceded by a decline of the [...] creased heart rate and contractility) it must [...] some functions in this process Cyclic GMP [...] 6-phosphofructokinase (EC 2 7 1 11) (Beitner [...] 1977) This effect is most pronounced when [...] enzyme is already partially inhibited by ATP [...] citrate e g under conditions in which the e[...] situation is good Cyclic GMP also abolish[...] antagonistic action of cyclic AMP and other a[...] teric activators such as glucose 1 6-bisphos[...] or AMP on the ATP or citrate inhibited [...] (Beitner et al 1977) In addition bovine lung [...] GMP dependent protein kinase has been sho[...] phosphorylate many glycogen degrading and [...] thetizing enzymes (Lincoln & Corbin 1977) [...] suggest that cyclic GMP in addition to the [...] of the reduced cytoplasmic calcium concentra[...] can mediate the metabolic effects of ACh and [...] ance the energy production to a lower level co[...] sponding the reduced energy demand

This research was supported by a grant from The [...] and Medica Scientific Foundation Finland. We are grateful to Mrs Si kka Helin and Mrs Vappu Koll[...] for Skilful technical assistance

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cts of nine different gastrointestinal polypeptides /ascular smooth muscle in vitro

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HELLSTRAND P & JÄRHULT J Effects of 9 different gastrointestinal polypeptides on vascular smooth muscle in vitro Acta Physiol Scand 1980 110 89-94 Received 27 Dec. 1979 ISSN 0001-6772. Department of Physiology and Biophysics, University of Lund, S eden

Nine polypeptides of gastrointestinal origin were tested for their possible effect on ascular smooth muscle of the rat portal vein. The substances tested were bombesin, caerulein, glucagon insulin, pentagastrin secretin, somatostatin, substance P and vasoactive intestinal polypeptide (VIP). Cumulative dose-response relations of integrated mechanical activity (mean tension) ere obtained ith maximal concentrations of the various peptides of 1–10 μg/ml Within this concentration range only substance P and VIP showed clearcut effects; substance P causing contraction and VIP relaxation The dose of substance P needed to produce contraction as high (ED_{50}>1 μM) so that the physiological importance of this response is doubtful. On the other hand, ED_{50} for the relaxing effect of VIP was about 15 nM which is in accordance with concentrations reported to produce significant vasodilatation in vivo The results support the ew that ascular effects which have been reported to occur in response to the other 7 peptides are mainly of indirect origin and not mediated via direct action on vascular smooth muscle

Key ord vascular smooth muscle, polypeptides, bombesin, caerulein glucagon, insulin, pentagastrin, secretin, somatostatin, substance P VIP

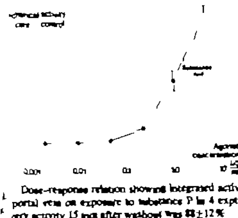
eral of the gastrointestinal polypeptides have ascribed effects on blood flow through many erent vascular beds (e.g. Thulin 1973 Bertaccini 6) However in many of these previous studies peptides have been infused in large doses and the effects on blood flow distribution have been her inconsiderable These findings suggest that blood flow responses represent pharmacological her than physiological effects or that the peptides on the blood vessels via indirect mechanisms.

The ascular smooth muscle of the rat portal vein plays spontaneous myogenic activity and the ctrical mechanical and metabolic properties of preparation have been characterized in detail g. Ljung 1970 Hellstrand 1979). In the present estigation w have used the portal vein to test e different mammalian polypeptides with regard their possible vasoactive properties. The study s shown that only two of them are potent vasoac e substances in itro viz. VIP and substance P

METHODS

Portal veins were dissected from male Sprague Dawley rats and suspended in organ baths made from glass syringes (plunger removed) mounted vertically in stirred water at 37°C. Two or four muscles were used simultaneously each mounted in separate syringe The muscles were connected to Grass FTO3 force transducers and kept at preload of 5 mN The syringes were filled with 4 ml of Krebs solution of the following composition in mM. NaCl 122, KCl 4 73 $CaCl_2$ 2 50 $MgCl_2$ 1 19 $NaHCO_3$ 15.5 KH_2PO_4 1 19 glucose 11.5 $CaNa_2$-versenate 0 026. The solution was bubbled with mixture of 96% O_2 and 4% CO_2, giving pH of 7.3–7 4

After an equilibration period of about 45 min, the expts were performed according to the following protocol. First, spontaneous activity of the portal vein was recorded for 10 min (=control period). The peptide to be tested, dissolved in 0.2 ml Krebs solution, was then added cumulatively in ascending doses. Each dose was allowed to act for 10 min and the volume in the bath was adjusted to 4 ml before addition of the next dose In most expts. 6 doses were given, differing each by a factor of 5 After the response to the highest dose had been recorded, the bath was rinsed twice and the muscle was left to recover for



... Dose-response relation showing integrated activi... portal vein on exposure to substance P in 4 expts. ...ery activity 15 min after washout was 88 ± 12 %

This strong excitatory response to substance ...s fully reversible.

...active intestinal polypeptide (VIP)

...3 illustrates original recordings of spontaneous ...ity of a portal vein in response to three differ... doses of VIP. This peptide caused a marked ...ction of the amplitude of spontaneous contrac..., while the frequency of contractions was little ...cted or somewhat increased.

...g. 4 sets out the compiled results from 4 expts ...hich the effects of VIP on mechanical activity ... tested. The two records marked ... were ob...ed with freshly dissolved VIP, whereas records ...ere obtained with peptide that had been frozen ... a few hours (see Methods). With freshly dis...ed VIP a dose of 1 µg/ml invariably caused ...mplete cessation of spontaneous activity. The ...ect of high doses of VIP were not completely ...ersible since activity after washout of the peptide was only about 60 % of that obtained during the initial control period.

Somatostatin

Fig. 5 shows that somatostatin caused a slight depression of mechanical activity to about 75 % of the control value at the highest dose. The recovery control activity was only 90 % of the initial activity so it is possible that the apparent depression of activity by somatostatin is in part due to fatigue.

Bombesin, caerulein, glucagon, insulin, pentagastrin and secretin

Figs. 6 and 7 show that none of the 6 other peptides tested had any significant effect on the mechanical activity of the rat portal vein. At high doses caerulein and secretin caused a slight excitation, whereas bombesin, pentagastrin and insulin caused some inhibition. The response of the muscles to glucagon was indifferent. Except after caerulein and secretin, the control activity at the end of each expt was reduced to 55–75 % of the initial value indicating that some of the peptide-induced inhibition of activity was not reversible and/or that some of it was due to fatigue.

DISCUSSION

Of the 9 polypeptides tested in this study, only substance P and VIP had a marked effect on the mechanical activity of the rat portal vein. The constrictory action of substance P noted here is in accordance with reported effects on intestinal and respiratory smooth muscle in vitro (Euler & Gaddum 1931, Pernow 1960, Nilsson et al. 1977, Cocks & Burnstock 1979), but contrasts to its vasodilatory

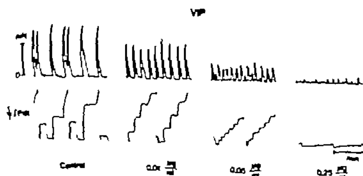


Fig. 3. Effect of VIP on mechanical activity of portal vein. Records taken as in Fig. 1.

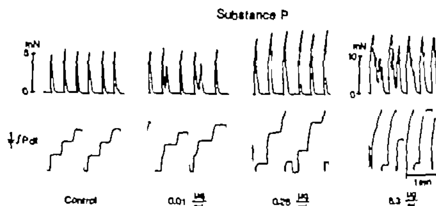


Fig 1 Effect of substance P on mechanical activity of the rat portal vein Records from last 2 min of 10 min exposures to the concentrations indicated Upper records active isometric tension Lower records integrated active tension over 1 min intervals in arbitrary units Preload 5 mN

15 min Recovery control activity was then recorded during 5 min

In all expts the mechanical activity of the portal vein was recorded with electronic integration of the tension in 1 min periods The mean output from the integrator during the whole initial period was used as the control value of mechanical activity whereas the mean integrated activity in response to the actual peptide was calculated from the values obtained during the final 5 min of each peptide period Dose response curves were then constructed by setting the control activity of each muscle at 100%

All peptides were kept frozen except for insulin which was refrigerated When available as pure substance the peptides were dissolved in saline either freshly on the day of the expt or prepared as stock solutions which were immediately frozen On one occasion a peptide (VIP) had to be frozen after an expt and used again the following day which resulted in a marked loss of activity (see Fig 4)

The following peptides were used Bombesin (Bachem) caerulein (Farmitalia) glucagon (Novo) insulin (Vitrum) pentagastrin (ICI) secretin (GIH Research Unit Karolinska Institutet Stockholm) somatostatin (Serono) substance P (Beckman) and VIP (generously supplied by Prof V Mutt GIH Research Unit Karolinska Institutet Stockholm)

Doses given are expressed as µg/ml final concentration except in the case of insulin where the concentrations are expressed as IU/ml For secretin conversion factor of 3 4 clinical units per µg was used (Vagne et al 1968)

Results are given as mean values ±S E.

RESULTS

Of all the substances tested for their effect on the rat portal vein (bombesin caerulein glucagon insulin pentagastrin secretin somatostatin substance P and vasoactive intestinal polypeptide (VIP) only substance P and VIP had prominent effects on isometric tension Dose–response relations for each peptide were obtained with activity of the portal veins quantitated as integrated (mean) active tension To test for possible effects of fatigue of the muscle Krebs solution was administered to control muscles according to the same protocol as in the expts with active peptides At the time of addition of the sixth dose activity was 90±7% of the initial value indicating that a reduction in activity of about 10% may be expected on the basis of fatigue alone

Substance P

Fig 1 shows representative recordings of the effects of 3 different concentrations of substance P on isometric tension of the portal vein Below the tension records is shown the output from the electronic integrator giving the time integral of tension in 1 min periods The recordings show the mechanical activity during the last min of the 10 min period that each dose was allowed to act on the preparation As can be seen substance P contracted the portal vein at all doses tested However even at the highest dose (6 3 µg/ml) a fully fused tetanic response such as occurs e g with high doses of noradrenaline did not appear The excitatory effect of substance P on the portal vein was not affected by phenoxybenzamine (1 µM) or atropine (10 µ Generally full effect was seen in about 1 min after addition of the peptide and the effect was well maintained during the whole 10 min period

Fig 2 summarizes the results from 4 expts with substance P As can be seen substance P at doses exceeding 0 1 µg/ml caused marked increments in mechanical activity At the highest dose (6 3 µg mechanical activity w about 7 t the co

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Dose–response relation showing integrated activity of portal vein on exposure to substance P in 4 expts. ...ery activity 15 min after washout was 83±12%

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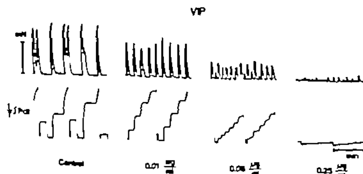


Fig. 3. Effect of VIP on mechanical activity of portal vein. Records taken as in Fig. 1.

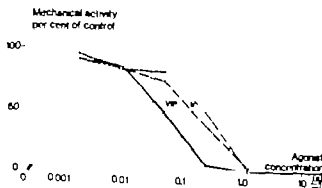


Fig 4 Dose-related effect of VIP on portal veins. Data from 4 individual vessels shown Records marked *a* show results with freshly dissolved VIP whereas records *b* were obtained using VIP which had been frozen after a few hours Mean recovery activity was 62±8%

action in vivo (Euler & Gaddum 1931 Löfström et al 1965 Hallberg & Pernow 1975 Pernow & Rosell 1975 Kaneto et al 1978) However no dilatory action of substance P on isolated vascular smooth muscle has been reported so far In the rabbit aorta it produces contraction (Moore 1979) and we have got unpublished data to demonstrate that it contracts the rat aorta as well Thus the constrictory action of substance P on the rat portal vein is not an exceptional response Certainly the concentrations of substance P needed to produce contraction in vitro (Fig 2) are much larger than those producing vasodilatation in vivo (e g Hallberg & Pernow 1975 Kaneto et al 1978) No "maximal" response to substance P was obtained with the concentrations used here (Fig 2) indicating that ED_{50} for its constrictory effect on the portal vein in vitro is well above 1 3 μg/ml (~1 μM)

The relaxing effect of VIP on the spontaneous activity of the rat portal vein had an ED_{50} of about 0 05 μg/ml (~15 nM) see Fig 4 This value is well in accordance with the plasma concentrations of VIP that have been shown to produce significant vasodilatation in vivo (e g Said & Mutt 1970 Kachelhoffer et al 1974 Eklund et al 1979 Järhult Hellstrand & Sundler 1980) but the value is higher than the portal concentrations of VIP observed after vagal stimulation (Schaffalitzky de Muckadell Fahrenkrug & Holst 1977 Fahrenkrug et al 1978 Bloom & Edwards 1980) The latency in onset of the VIP effect has been a consistent observation both in vivo and in vitro (e g Kachelhoffer et al 1974 Cocks & Burnstock 1979) but no information is available regarding possible mechanisms underlying this phenomenon In the study by Järhult Hellstrand & Sundler (1980) it was shown ... significantly reduced the vasoconstriction ... by sympathetic stimulation Further work ... sary to elucidate the effects of VIP both ... to depression of myogenic tone and to ... teraction with excitatory agents.

Several of the other peptides studied ... been proposed to influence vascular tone ... stance somatostatin has been claimed to ... splanchnic and hepatic blood flow in man ... (Wahren et al 1977 Samnegård et al. 1979 ... et al 1979) As seen from Fig 5 we found ... relaxing effect of somatostatin on the portal ... but it is certainly very slight in comparison ... VIP Also it appears as if the doses used ... low compared to those required to give a ... ble effect on the portal vein This would ... indirect action of somatostatin on vascular ... ance

Of the remaining six peptides tested (Figs ... 7) bombesin and insulin were the only ones ... ing any degree of relaxation of the portal ... However it is highly unlikely that these ... may physiologically be present in concent... large enough to produce such effects ... Caerulein is closely related to cholecys... which like secretin and pentagastrin produce ... moderate increase in intestinal blood flow (Fr... al 1973) and interestingly in human ... der blood flow (Ihse & Järhult unpubl...) Glucagon too has repeatedly been shown ... duce vascular resistance especially in ... cular beds in the gastrointestinal tract (... et al 1959 Ross 1970 Tibblin et al 1970 ... & Mailman 1977) and an antagonizing ... glucagon on the sympathetic vasoconstrict...

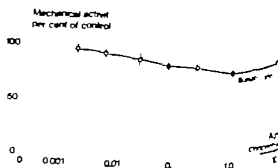


Fig 5 Mechanical activity of portal vein on exposure to different concentrations of somatostatin Recovery ... ty was 90±10%

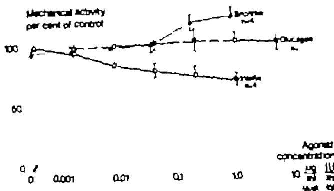


Fig. 6. Mechanical activity of portal veins on exposure to different concentrations of secretin (μg/ml), glucagon (μg/ml), and insulin (IU/ml). Recovery activities after washout were for secretin 90±4%, for glucagon 74±8% and for insulin 57±6%.

...patic vascular bed has also been reported ...rdson & Withrington 1977). However the ...t results from the portal vein show no effect ...cagon on this isolated vascular smooth mus... should be noted that the doses of glucagon ...lly used in the in vivo expts referred to above ...ceed those obtained during physiological con...

...results show then, that of the 9 peptides ...l only VIP is able to directly influence vascu... ...ooth muscle tone in vitro in a way that seems ...atible with reported in vivo effects of the sub... Any vascular effect mediated by the other ...des studied is probably produced by indirect

...tudy was supported by grants from the Swedish ...al Research Council (04X-00028 and 04X-2210), the Medical Faculty, University of Lund, and AB Hässle, Göteborg. We especially acknowledge the skilled technical assistance provided by Monica Lundahl, Monica Heidenholm and Ina Nordström.

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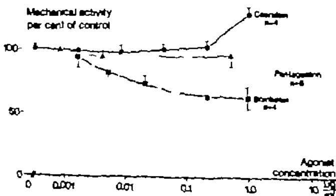


Fig. 7. Mechanical activity of portal veins on exposure to caerulein, pentagastrin and bombesin. Recovery activities after washout were for caerulein 103±4%, for pentagastrin 72±7% and for bombesin 76±10%.

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·ous tissue osmolality water and electrolyte ·sport in the cat small intestine at varying ·inal osmolalities

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HALLBÄCK, D.-A., JODAL, M. & LUNDGREN O. Villous tissue osmolality water and electrolyte transport in the cat small intestine at varying luminal osmolalities. Acta Physiol Scand 1980, 110.95–100. Received 27 Dec. 1979. ISSN 0001-6772. Department of Physiology University of Göteborg, Sweden.

Villous tissue osmolality and net transport for water sodium, pota sium and chloride were determined in the feline small intestine when exposing the mucosa to solutions with different mannitol concentrations (0 100 315 and 600 mmol/l). Tissue osmolality at the villous tip aried with luminal osmolality. At the villous base on the other hand, tissue osmolality remained around the plasma osmolality regardless of the osmolality of the luminal fluid. Transport rates for water were affected in the way predicted from the lumen to tissue osmolality difference. A net flux from tissue to lumen was alw y recorded for the studied electrolytes. The hydraulic conductivity (L_p) of the intestinal epithelium with dilated intercellular spaces was estimated from the present result to be around 30 10^{-14} cm Pa. When the intercellular spaces were collapsed L_p was estimated to be 15 10^{-} cm s^{-1} Pa

Key ords Intestinal water transport intestinal electrolyte transport, hydraulic conductivity of intestinal epithelium

ransfer of water from the intestinal lumen into ·llous tissue is in vi accomplished by the ·ction of a hyperosmolar compartment in the core secondary to the action of the intestinal countercurrent multiplier (Haljamae et al 1973 et al. 1978). The tissue osmolality at the tip of ·llus has been estimated to about 1 000 mOsm/ ·O in cat (Jodal et al 1978) and to about 800 ·m/kg H_2O in man (Hallback et al 1978) when ·sing the intestinal mucosa to an isotonic sol· containing sodium and glucose

·n previous study (Hallback et al 1979) it was ·n that varying the sodium and/or glucose con·ration in the luminal solution altered the tissue ·lality in a manner consistent with known cellular transport mechanisms in the enterocytes. The ·tions in tissue osmolality influenced in turn the ·ater uptake from the gut lumen. In the present the osmolality in the intestinal lumen was ·ied by posing the mucosa to different mannitol solutions, in order to analyze the effect of such ·ges on villous tissue osmolality and net transport of ater and electrolytes.

METHODS

A *Operat procedures*

The periment were performed on cats anesthetized ·ith chloralose (50 mg/kg b w) after ther induction. The animals had been deprived of food for 4 h and had no obvious signs of intestinal infection. The operati procedure were largely similar to those described by Jodal et al. (1975) which paper should be consulted for details. Mean arterial pressure was measured from the left femoral artery by pressure transducer (Statham P23AC). The splanchnic nerves were divided bilaterally and the cholinergic influence was eliminated by atropine administration, 1 mg/kg b.w Acidosis was prevented by slow continuous infusion (0 1 ml/min) of bicarbonate-glucose solution in the left femoral ·ein (Haglund & Lundgren 1972)

B *Recording of intestinal net ater transport and experimental procedures*

Net water absorption or secretion was determined according to the method described by Jodal et al. (1975) Four segment were used in randomized manner A recirculating ystem was coupled to the lumen of each intestinal segment. The volume change of this ystem was continuously recorded with volume transducer connected to the ystem via Y-tube Provided no motility occurred the recorded volume changes reflected net water absorption

Table 1 *Net rates of water and electrolyte transport in the jejunum of cats when exposing segments to four solutions with different concentrations of mannitol*

(−) denotes a transport from tissue to lumen Mean ± S E.

Mannitol conc (mmol/l)	Water µl×min ×100 cm	Sodium µmol×min ×100 cm⁻²	Potassium	Chloride
0 (n=6)	−58± 6	−34 1± 5 8	−1 46±0 41	41± 6
100 (n=7)	37?±?6	−20 ± 5 9	−1 3?±0 19	−37+ 8
315 (n=7)	60±36	−19 0± 7 3	−0 79±0.23	−29 8
600 (n=5)	−158± 5	−28 7±11.5	−0 69±0 19	−39+1

Statistically significant from 315 mmol/l

or secretion The intraluminal pressure at the outflow end of the segment was kept at 1 cm H_2O and the intestinal segment was perfused at a constant rate of 1 ml/min by means of a roller pump (Model mp-4 Ismatec Sa Zurich Switzerland) Recirculation was prevented by including a large reservoir (700–1 000 ml) in the circulating system The temperature of the perfusate entering the segment was continuously monitored with a thermocoupled thermometer (Electrolab Copenhagen) and kept at 38°C by a heating pad The temperature close to the intestinal segments was kept constant by a lamp connected to a temperature controlled relay

After a perfusion period of 30 min samples of the perfusate entering and leaving each segment were collected Immediately thereafter the segments were rapidly extirpated and momentarily frozen in isopentan precooled to about −150°C in liq id nitrogen

C *Biocl emi l and o motic measurem nt*

In order to calculate the net transport of electrolytes across the intestinal epithelium samples (about 5 ml) were taken from the perfusates entering and leaving the intestinal segments The samples were collected in plastic tubes and the sodium and potassium concentrations were determined with a flame photometer (Eppendorf) while the chloride concentration was determined with a chloride meter (Corning Eel 9?0 Chloride Meter Hal tead Essex England) The osmolalities f the samples were determined with an osmometer (Advanced Instruments Inc model 68-311AS).

Intestinal net transport of sodium pota sium and chloride was calculated as described by Hallback et al (1979*b*) Net transport rates were related to serosal rea and expressed as µl/min ×100 cm² serosal urface (wate) or as µmol/min ×100 cm serosal urface (electrolyte)

D *Cry copic technique*

1 *General proced re* The cryoscopic method with calibration procedure was presented in detail by Jodal et al (1978) Briefly the frozen intestinal segment wa cut obliquely i a cryostate kept at −25°C so as t gi e 15 µm thick cross-sections at different level along the villus. The tissue sections were embedded in kerosene between glas plates and transferred to a temperature-controlled unit consisting of an aluminium block mounted on a Zei standard microscope connected with an automatic (Robot Star 50) The temperature of the aluminium could be set at any temperature between − +0 5°C Thawing of the intestinal tissue was achieved stepwise increases of tissue temperature which automatically with a 5 min equil bration period for each step At the end of each period the tissue was photographed

2 *Determination of t ssue osmolality* The temperature at which the last ice crystals thawed in a given section was taken as its melting point The tissue melting point determined in this study was considered a measure of the villous core osmolality The magnification used (about 50 times) was too low to allow any detection of intracellular ice crystals in the villous tissue percentage of melted villous tissue for each level was determined from the photographs The were fed into a computer which calculated the osmolality at 5 10 20 30 40 50 60 70 80 90 of length as well as the mean osmolality between 5 and and 50 and 5 and 100% illous length as described in detail by Jodal et al (1978)

E *Histological proced re*

In three experiments a rectangular piece (×3 cm) perfused ntestinal segment wa mounted on cork fixed n 10% neutral formaline The block were for routine paraffine embedment cut at about mou ted and sta ned according to v Gieson

F *Solution*

The intestinal mucosa was exposed to mannitol of four different concentr tions i e 0 100 315 mmol/l In all experiment only one type of solution perfused through each segment

G *Stat tic*

Statistical significance was tested using the sign Wilcoxon nonparametric test (Siegel 1956)

RESULTS

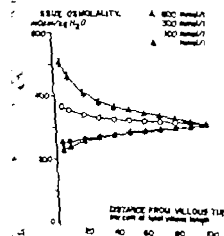
Table 1 gives the results on net water and electrolyte transport rate in the different types of

_ T p and mean osmolality n rious portions of intestinal villi expressed in mOsm×kg H_2O Mean

	Tip	From 5 to 30 % of villous length	From 5 to 50 % of villous length	From 5 to 100 % of illous length
(6)	23_± 9	254 8	265_ 7	282 6
(7)	253± 9	271±13	272± 8	283 4
70)	369± 9	346 7	338± 6	327_4
17)	507 1	430 11	403±10	367±6

eols The expected decrease in net water e with increasing mannitol concentration in minal perfusate is evident, although the water rption rate from solution devoid of mannitol ower than that from a 100 mmol/l mannitol on. The electrolytes studied all exhibited a net from tissue to lumen. Sodium and chloride port rates showed no consistent change when ng the luminal perfusate osmolality while the ss of potassium from the tissue decreased with easing lumen osmolality

e villous core osmolality as estimated with the scopy method is shown in Table (villous tip olality and mean osmolality at varying levels g the villi) and in Fig. 1 (osmolality gradient in ril) The exposure of the intestinal mucosa to wo hypotonic solutions produced a villous tis hypotonicity particularly in the villous tips, while osmolality approached isotonicity in the basal villous portions. On the other hand with the hyper tonic mannitol solution in the intestinal lumen tissue osmolality increased in the upper villous por tions.



1 Villous tissue osmolality when varying the man concentration of the solution perfusing the intestine denote S.E. At many points the S.E. is so small that its than the area of the symbol For number of rvations, see Table 2.

Knowing tissue and luminal osmolality it was possible to estimate the transepithelial osmotic gradient for each expt In Fig. the lumen to tissue osmolality difference is plotted against net water transport rate for all groups except the one with no mannitol When calculating lumen osmolality a mean value of the entering and leaving fluid osmolality was estimated assuming an exponential change in the intestinal segment (Soergel et al 1968) In this figure a corresponding calculation is also included using the values reported by Hallbäck et al (1979b) In that study the tissue osmolality was varied by perfusing the intestinal lumen with isotonic solutions of different sodium and glucose concentrations.

The values for tissue osmolality were those calculated between 5 and 30 % of villous length (Table) as water absorption was assumed to occur mainly across this part of the villus. The results of the two studies form two parallel biphasic curves that of the present study being placed to the left of the one of Hallbäck et al (1979b) The two phases of the curve based on the results of Hallbäck et al were constructed using the methods of least squares. These lines are indicated in the Figure

The slope of the curves represent the amount of fluid transported per unit osmolality difference i.e. they reflect the hydraulic conductivity (L_p) of the intestinal epithelium. In table 3 the corresponding hydraulic conductivities are calculated assuming a reflection coefficient of 1 for mannitol and sodium chloride the latter probably being the main solute in intestinal interstitial fluid Water absorption is assumed to take place across the upper third or half of the villus The corresponding values of the ex

Table 3 *Hydraulic conductivity (Lp) of the intestinal epithelium in cm ×s ⁻¹×Pa ⁻¹ as calculated two slopes of the curve illustrated in Fig 2*

The Lp values have been estimated assuming water absorption to occur across the upper third or half of the ?

	Upper 30 % of villus length		Upper 50 % of villus length	
	Steep slope	Flat slope	Steep slope	Flat slope
Present study	31×10	14×10	19×10	8×10
Hallbäck et al (1979*b*)	6×10	18×10	.2×10	16×10

periments reported by Hallbäck et al (1979*b*) are also included in Table 3

The histological investigations revealed damaged epithelial cells restricted to the very tips of most villi in intestinal segments exposed to solutions containing no mannitol and in some villi in the 100 mmol segments No histological damage was observed in segments perfused with 315 or 600 mmol mannitol solutions

DISCUSSION

The present study demonstrates that varying the osmolality of the intestinal lumen within wide limits in some way affects markedly the tissue osmolality in upper parts of the villus At the base of the villus on the other hand tissue osmolality approached that of arterial plasma regardless of which fluid that was placed in the intestinal lumen Hence the plasma osmolality in the vessels draining the intestinal villi is always close to isotonicity This considered as one functional effect of the countercurrent exchanger (Lundgren 1967) the subepithelial capillaries/veins in the villus equilibrates with the isotonic blood in the situated supplying arterial vessel Had the larles/veins been located at some distance from supplying arterial vessels the venous plasma molality had much more closely reflected that intestinal contents Hence the intestinal countercurrent exchanger acts as a damping device respect to the luminal osmolality a function must be of particular importance in the duodenum

The presence of osmoreceptors in the mucosa which influence the rate of gastric emptying has been inferred from a large variety of experiments (cf Hunt & Knox 1968) The demonstration in this and previous studies (Jodal et al 1 Hallbäck et al 1979) that the intestinal counter sodium and/or glucose or the intestinal lumen

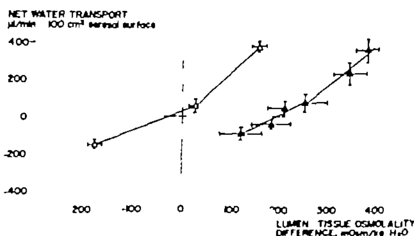


Fig 2 The relationship between net water transport and lumen to tissue osmolality difference The right curve was constructed from the results reported by Hallbäck et al (1979*b*) using the method of least squares The steep part of this curve was calculated from the three highest osmolality difference values and the flat part from the four lowest values Bars denote S E For number of observations see Table 1 and Hallbäck et al (1979*b*)

particularly influence villous osmolality ggest that the osmoreceptors for gastric g are located in the core of the villus. This sis has, however never been tested since l osmolality have not been determined in the ations of gastric emptying.

illustrates that net water transport across stinal epithelium is not linearly related to the ed lumen to tissue osmolality difference henomenon has been demonstrated for sev ithelia and has been named rectification of water flow As discussed by House (1974) st likely explanation is the changes in mor y of the intestinal epithelium induced by al the direction of net water flow When the tissue is hypertonic in relation to lumen and onsequently is into the tissue the lateral in llar spaces are distended (Tomasini & Dob 70 Dibona et al 1974). When water moves in posite direction the intercellular spaces col offering increased flow resistance (right part 3 in McElligott et al 1975). Based on this ing it is proposed that the hydraulic conduc during absorption is represented by the de e of the upper teeper curves in Fig. . Simi the L_p value of the intestinal epithelium with sed intercellular spaces is reflected in the de e of the two lower curves of Fig (see also 2). It is further proposed that the L_p value net water absorption, i.e. about 30×10^{-11} cm Pa reflects almost exclusively L_p of the unctions This proposal is based on two as ions. (1) In this situation water enters the xclusively through the tight junctions and (2) istended parts of intercellular spaces do not ny substantial flow resistance compared with ght junctions.

overall hydraulic conductivity of the intesti pithelium has been measured with a method w to one used in this study by Fordtran et al) and Soergel et al (1968) although these au considered the villous tissue osmolality to be ue Recalculating their results to the unit for sed in this study and using the anatomical nsions for the human intestine reported by gel et al (1968) one arrives at the values of 34 16 10^{-} cm $\times$Pa in the two abovemen d investigations. These values were based on riments where a mannitol induced net secre of fluid was present (right part of Fig. 3) and ld consequently be compared to the value around 15×10^{-11} cm$\times$s $\times$Pa found in the present study

The curves relating lumen-tissue osmolality difference to net water transport (Fig. 2) do not coincide the curve of the present experiments being located to the left of that constructed from the results of Hallbäck et al (1979*b*) It is not possible from the present results to explain this difference between the two curves, but at least two contributing factors exist. First, the reflection coefficient of mannitol (in the lumen) is probably higher than that of sodium chloride (cf Fordtran et al 1965) which constitutes most of the hyperosmolality in the intestinal villi Second in the experiments of Hallbäck et al an increased water flux from tissue to lumen in e.g. the crypts may explain why their curve is placed more to the right.

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h concentration of immunoreactive calcitonin rostatic origin in human semen

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tly it was demonstrated that only about 4% calcium in human semen is present in ionized Arver & Sjöberg 1980). The fact that seminal ncentration is about 3 times that of blood suggest the existence of a mechanism for ntration of Ca in seminal fluid. Calcitonin might play role in this connection. For this and also since CT is known to increase the xcretion of Ca (Hjern Granberg & Sjöberg w measured the apparent CT concentration man semen to see whether it might be positi e related to Ca concentration

men samples were obtained from 18 men at g the reproductive physiology unit for a bar tion In addition, sperm free seminal fluid was ted from 4 vasectomized men and split ejacu- from 6 volunteers (medical students) 13 of the n samples with normal recording concerning morphology and seminal plasma composition ra according to Eliasson 1975) were regarded reference group which as compared to 5 n samples with apparently subnormal prostatic ibution (zinc <1 mM) and to the samples the asectomized men having normal com- ion of the seminal plasma In another series of riments, split ejaculates (Kvist 1980) were employed to obtain semen fractions with either pre dominantly prostatic (portion 1) or vesicular gland (portion) contributions to the seminal plasma.

CT was measured by radioimmunoassay using rabbit antibodies to synthetic human calcitonin (Immuno Nuclear Corp USA) Normal human serum CT measured with this method had given a mean value of 4 pg/ml (range <5–100) Ca Mg and Zn were determined with atomic absorption spectrophotometry acid phosphatase activity and fructose concentration with colorimetric methods (cf Eliasson 1975) For descriptive statistics mean and S D are given. Correlation ratings were est mated as Pearson s product moment correlation. Student s t-test was used for group and paired analyses (cf Colton 1974)

In the reference group mean seminal plasma CT was 1771 pg/ml (range 1000–2880 S D ±612) i.e about 40 times serum CT The values for other semen variables are presented in Table 1 CT was positively correlated to Ca, Mg Zn and acid phos- phatase ("prostatic markers") but not to fructose (" esicular gland marker") (Table) Furthermore subdivision of the reference samples into a low (<16 mM) and high (>16 mM) fructose group did not reveal any CT difference between the two

e 1 *Concentrations of calcitonin total al- magnesium zinc acid pho phatase activity fructose in seminal plasma f om 13 subject*

	Mean ± S D	Range
itonin, pg/ml	1771±612	1000–2880
ium, mmol/l	6 1 1 6	3 4–8 7
nesium, mmol/l	4 ±1 27	2 0–6 9
mmol/l	5±1 0	1.3–4 6
phosphatase al/l	14 9±7 1	7 7 33.9
tose mmol/l	15 7±6 3	7.3–30 8

Table *Correlation coefficient between calc tonin nd ecretory products f prostat nd sicular origin*

$N = 13$

Correlation to		P	Origin
Calcium	0 63	<0 001	Prostatic
Magnesium	0 77	<0 001	Prostatic
Zinc	0 80	<0 001	Prostatic
Acid phosphatase	0 75	<0 001	Prostatic
Fructose	0 16	<0 2	Vesicular

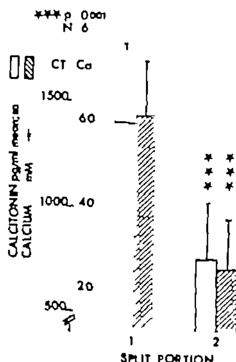


Fig 1 Calcitonin and calcium concentration in portions 1 ("prostatic fluid") and portions ("vesicular fluid") of split ejaculates of human semen

The group of semen samples with subnormal concentrations of Zn and other prostatic markers contained significantly less CT than the reference group ($P<0.01$ df=16) In contrast seminal fluid from the vasectomized men contained as much CT as the reference group and also had normal concentrations of Ca Zn and other prostatic markers

The split ejaculate experiments revealed obvious differences between portions 1 and 2 In portion 1 ("prostatic fluid") the concentrations of CT Ca (Fig 1) Mg Zn and acid phosphatase activity were conspicuously higher ($P<0.001$ $N=6$) than in portion 2 ("vesicular gland fluid")

DISCUSSION

The present demonstration of high CT immunoreactivity in human semen may provide an explanation of the steep gradients between blood serum and semen Ca and between ionized Ca and total Ca in the seminal plasma (Arver & Sjöberg 1980) The presence of large amounts of CT somewhere in the male accessory glands may be the ultimate cause of the conspicuous Ca transfer to the seminal fluid and may also explain why CT per se is also transported in large amounts to this fluid Here the hormone may counteract the ionization of Ca and influence the exchange of Ca be[tween] spermatozoa and their external en[vironment] possibility that seminal plasma CT mi[ght] from a higher level of the male reproduc[tive] (testis/epididymis) seems to have been [...] by the observation that also ejaculates fr[om vasec]tomized men contained as much CT a[s refer]ence semen samples

CT has been found to stimulate not only [...] but also renal Mg excretion (cf Munson [...] the present study seminal CT was corre[lated] to Mg and Zn Therefore CT may faci[litate not] only the transfer of Ca but also of other [...] cations to the seminal fluid

Regarding the source of seminal CT the [...] experiments strongly suggest that the ho[rmone is] added to the semen by prostatic secretion [...] determinations revealed a positive correl[ation be]tween the concentrations of CT and prosta[tic mark]ers in semen Furthermore in the split e[jaculate] experiments the concentration of CT was [consid]erably higher in the fractions of the ejacula[te domi]nated by prostatic fluid than in those co[nsisting] predominantly of vesicular gland fluid How[ever it] remains to be elucidated whether CT might [be pro]duced by some cells in the prostate gland [or if the] high amounts of CT in its fluid is a manifest[ation of] prostatic trapping of circulating CT

We are greatly indebted to Drs R. Th[...] Löw for generously supplying us with laboratory [...] Financial support was obtained from Karolin[ska Ins]titets Fonder

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›s the central pattern generation for locomotion .ımprey depend on glycine inhibition?

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:nowledge about the general organizational :s underlying the neural control of locomo- vertebrates has expanded markedly during ıt few years (cf Stein 1978 Shik & Orlovsky Grillner 1975) On the other hand the exact ıral circuitry controlling locomotion or any ımate rhythmic behaviour is still unknown le vertebrate networks are required to eluci- how such circuits may operate Such mod- :an later be tested on higher vertebrates to find f they apply or how they may have become fied We have used one simple vertebrate aration the lamprey spinal cord in which the ›mic activity normally generating swimming be elicited also under in vitro conditions (Poon › Rovainen 1977 Cohen & Wallén 1978, Wallén ‹) Several modes of operation have been ›ested for such central pattern generators Gs) (Gurfinkel & Shik 1973 Kling & Székely ‹ Selverston 1976 Getting et al 1980) Some on burst generating circuits of excitatory ›rons, others exclusively on inhibitory neurons mixture of the two In order to discriminate be en the first and the last two possibilities the ›ent experiments were designed. As a result we come to seek evidence for the possible inhibi- transmitters involved Homma & Rovainen ›78) have shown that the putativ inhibitory ›mitters glycine and γ-amino-butyric acid ›ABA) (cf Krnjević 1974 and Martin et al 1970) ›g both act in the lamprey spinal cord. Strychnine ›ycine) and bicuculline (GABA) block their action ›n specific manner (cf Krnjević 1974 Homma & ›vainen 1978 Curtis et al. 1968). Both amino acids ›se a marked increase of membrane conductance ›, Cl⁻-ions, associated with a neuronal hyper ›larization (Homma & Rovainen 1978).

›he lamprey spinal cord is flat (200 μm thick, 2 mm ›de) and does not contain blood vessels in vivo as it is ›ygenated from the cerebrospinal fluid, which makes it ›uitable for in vitro experiments. The spinal cords of adult ›. Ichthyomyzon unicuspis ›ere dissected in pieces of 10–25 segments (notochord till attached) and placed in an in vitro chamber and pinned dow in "sylgard" The cord was superfused ith lamprey physiological solution (Wickelgren 1977) and the activity of the entral root was recorded with suction electrodes. After application of D-Glutamate (0.3–1 2 mM) in the bath, "fictive swimming starts (Poon 1980 Cohen & Wallén 1978 1979), i e. bursting motor output in each segment (Fig. 1 A) with ade quate intersegmental coordination The temperature was kept at 7–8°C The rate of bursting may be markedly de pendent on the pool temperature (Fig 1 F and Legend).

The frequency of the rhythmic alternating activi- ty (Fig. 1 A) increases if strychnine (0.5–3 μM) is applied in the bath (Fig 1 B and E) to become irregular and change over to tonic efferent acti ity with a higher dose (Fig. 1 C) As the rate of activity is modified strychnine must interact with the pat tern generator itself or the spinal neurons control- ling it. The amount of strychnine used is low (Fig. 1 B). It is in the lower range of doses used by Homma & Rovainen (1978) to demonstrate competetive an- tagonism between glycine and strychnine in this preparation. This finding thus suggests that neurons releasing the putative transmitter glycine are active in the pattern generation If so it would be ex pected that glycine itself should also influence the bursting. Glycine may cause both marked ir regularities in the burst pattern and a decreased rate (50 μM in Fig. 1 D). At somewhat higher dosages all acti ity ceases. Homma & Rovainen (1978) used concentrations of up to 1 000 μM glycine to obtain maximal conductance effects When applying glycine in the bath it will of course act on all neurons with glycine receptors The effect of strychnine on the other hand will only be mani- fested in those synapses which are active and re lease glycine in this particular experimental condi- tion (fictive swimming).

The GABA antagonist bicuculline should in the same way as strychnine affect the activity of the pattern generator if presumed GABA neurons were important in this context. Bicuculline (Fig A, B E) has no effect in a dosage which will block the

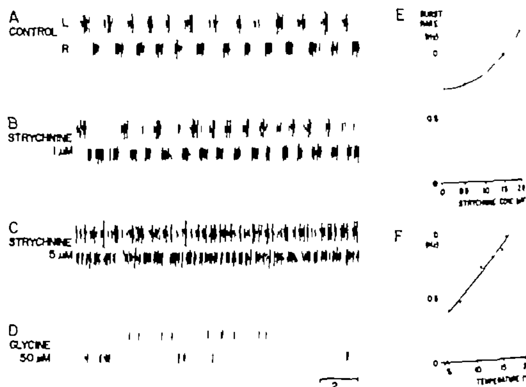


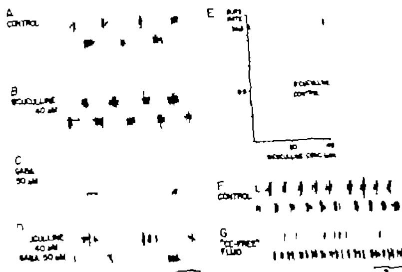
Fig 1 The effect of glycine and strychnine on the burst pattern during fictive locomotion A shows the burst recorded in one ventral root on the left (L) and one on the right (R) side when using normal physiological fluid mM glutamate added (control for B–D) In B and C 1 and 5 µM strychnine was added respectively D shows hibitory effect of 50 µM glycine Note time calibration The graph in E shows the burst frequency at different centrations of strychnine for a different preparation and F the effect of different bath temperatures on the In this graph the temperature was progressively increased from 5 to 20°C within 11 min The sensitivity to seems to vary i.a between preparations

conductance changes evoked by GABA (Homma & Rovainen 1978 see also Fig 2 D) Already at 1 4 µM bicuculline their GABA effects were antagonized It thus appears that synapses with receptors blocked by bicuculline are not of critical importance in producing this burst pattern Application of GABA to the bath can reveal if pattern generator neurons (including those controlling the CPG) have GABA receptors There is a marked reduction of the burst rate already with 50 µM GABA (Fig 2 C) This effect of GABA is antagonized by bicuculline (Fig 2 D) Neurons releasing GABA are thus apparently not of importance for the generation of the present fictive locomotion but if such neurons were to become active in some other behavioural condition they could influence the pattern generation

To further test the possibility that inhibitory mechanisms are involved in the pattern generation we have blocked the inhibition associated with a conductance increase to Cl ions (e g GABA and glycine Homma & Rovainen 1978) This can be accomplished by utilizing a physiological fluid in which the chloride content has been reduced by substituting Cl with SO_4^{2-} (Calabrese 19 Homma & Rovainen 1978) The application of a solution gives practically no effect on action potentials or on the membrane po (Homma & Rovainen 1978) Glycine does give rise to a transient depolarization but there is no conductance increase at all (presumably intracellular Cl -concentration approaches Application of such Cl free" physiological tion (with D-Glutamate) results in a complete down of the burst pattern and a tonic activity F G)

In conclusion neurons releasing a tra blocked by strychnine (presumably glycine an active part in producing the burst pat contrast possible GABA neurons seem no involved under the present experimental tions

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The effect of GABA bicuculline and chloride free physiological solution on the burst pattern during fictive tion. A shows the control burst pattern as in Fig. 1 (0 3 mM glutamate) and B, C and D the effect of bicuculline (40 ABA (50 µM) and the combined effect of bicuculline and GABA. The effect of GABA is clearly antagonized by line. The graph in E show the absence of effect of bicuculline on the burst rate at both low and very high doses. were measured just prior to the respective bicuculline application. F is the control burst pattern (0.3 mM ate) for G which shows the effect of applying Cl -free solution Time calibrations for A–D and F–G respec

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xistence of an avian pancreatic polypeptide (APP) unoreactive substance and catecholamines ome peripheral and central neurons

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eatic polypeptide (PP) was isolated by Kim t al (1971 1975) from chicken pancreas and ural characterization of avian PP (APP) re d that it is built up from 36 amino acids (see nel et al 1975) Recently Lorén et al. (1979) described a wide distribution of APP immuno- ve neurons in both the central and peripheral ous ystem.

docrine cells in the gastro-intestinal tract and peripheral tissues often contain both a mic amine and a polypeptide hormone (see se & Polak 1978) and this has recently been ob- ed also in neurons (see Hokfelt et al 1980). In earch for further examples of coexistence situ- we have examined in detail the distribution of in relation to some known transmitter related ms. In the present preliminary paper we report a peptide reacting with the APP antiserum can bserved in sub-populations of several types of cholamine (CA) neurons in rat and cat.

tibodies to APP were raised in rabbits (Kimmel et al. The antiserum was used in dilution of 1 100 APP erum absorbed with an excess of APP (50 μg/ml erum diluted 1 100) was used as control For de of antibodies to tyrosine hydroxylase (TH) and ylethanolamine N-methyltransferase (PNMT), two cholamine synthetizing enzymes purified from the rat ochromocytoma and rat adrenal glands, respectively Markey et al (1980) and Goldstein et al. (1978) Male to rats (Sprague Dawley b.wt. 150–200 g) and adult of both sexes were used. Two rats received an in- entricular injection of colchicine (60 μg in 20 μl). Two received two injections of 6-hydroxydopamine OH-DA) (100 mg/kg) 48 h and 24 h before sacrifice. In 2 the sciatic nerve was ligated 24 h before sacrifice In 3 the superior cervical ganglion was extirpated 14 days re sacrifice The experimental animals were perfused 10 % ice-cold formalin or fixed with modified nique (Lundberg, in preparation) and the brain, spinal cord and some peripheral tissues were dissected out. After further fixation by immersion and rinsing, consecutive cryostat sections were processed for the indirect im- munofluorescence procedure Briefly 3 consecutive sec tions were incubated with antibodies to TH APP and PNMT respectively at +4°C for 24 h, rinsed incubated with fluorescein isothiocyanate (FITC) conjugated sheep antirabbit antibodies for 30 min at +37°C, rinsed, mounted and examined in Zeiss fluorescence microscope. In some experiments the elution and restaining technique of Tramu et al. (1978) was used to establish coexistence of two antigens in the same cell bodies.

In the *periphe al nervous system of the rat* APP like immunoreactivity was observed in the majority of the principal sympathetic ganglion cells in the superior cervical the stellate and the celiac ganglia. APP immunoreactive nerve terminals were widely distributed. Thus APP immunoreactive plexuses were seen in high concentrations in the vas deferens (Fig. 1A) and in the heart, with particularly high amounts in the auricles In the submaxillary gland and the iris only few fibres were observed. On adja- cent sections the APP-immunoreactive cell bodies were also positive to TH (but not to PNMT) in the ganglia described above. Also nerve fibres contain- ing TH were seen in the submaxillary gland heart and vas deferens (Fig. 1B) with a similar distribu- tion pattern However so far many more TH than APP immunoreactive fibres were seen in the iris. Furthermore in the submaxillary gland the APP positive fibres were mainly seen in relation to blood vessels whereas the fibres containing TH in addi- tion were seen in the exocrine parenchyma. After 6-OH-DA treatment a marked decrease in the num- ber of both APP and TH immunoreactive fibres was observed in the submaxillary gland, heart and vas deferens (Fig 1C) After ligation of the sciatic

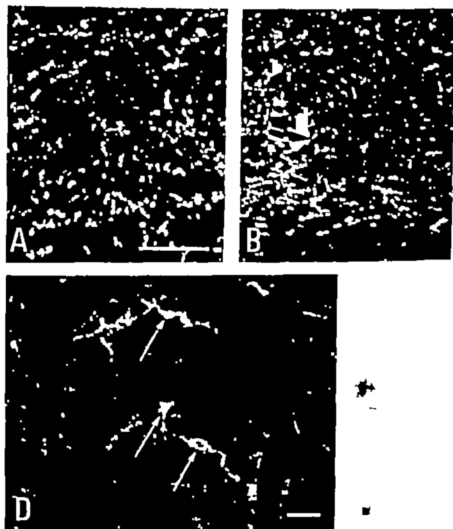


Fig 1A–F Immunofluorescence micrographs of the vas deferens (A–C) and ventrolateral medulla oblongata of untreated (A B) 6-hydroxydopamine (6-OH DA) (C) and colchicine (D F) treated rat after incubation with antiserum to APP (A C D) and tyrosine hydroxylase (TH) (B F) D and F show the same section (elution according to ... et al 1978) (A–C) Dense network of APP immunoreactive and catecholamine nerve terminals are seen in the vas deferens After 6-OH DA treatment most of the APP and TH (not shown) immunoreactive fibres disappear (C) Neuronal cell bodies (arrows) contain both APP-like immunoreactivity and TH Bars indicate 50 µm

nerve a marked accumulation of both APP and TH like immunoreactivity was observed mainly central to the ligature The accumulation of immunoreactivity exhibited a marked overlap when comparing the adjacent sections incubated with the respective antibody

In the *cat peripheral nervous system* the majority of neuronal cell bodies in the cervical stellate and celiac ganglia were both APP and TH immunoreactive No certain APP like immunoreactivity could be seen in the sphenopalatine ganglion cells which belong to the parasympathetic system APP immunoreactive fibres were found in most peripheral tissues including heart particularly auricles nasal mucosa vas deferens and in low amounts in the submandibular gland and iris TH immunoreactive fibres were seen on adjacent sections of the tissue with a rather similar distribution After removal of the superior cervical ganglion the APP and TH immunoreactive fibres in the nasal mucosa completely disappeared Both in the cat and rat APP like immunoreactivity was observed in a population of adrenal medullary gland cells

In the *rat central nervous system* APP immunoreactive neuronal cell bodies and nerve terminals exhibited a wide spread distribution as also partly described by Lorén et al (1979) Here some of the systems will be dealt with ... containing both this peptide and catecholamine synthesizing enzyme Elution and restaining

o Tramu et al (1978) revealed that cell mmunoreactive to both APP (Fig. 1 D) TH) and PNMT with identical distribution were observed mainly in two groups in the oblongata. (1) the nucleus tractus solitarii extending dorsally into the midline area of iculus longitudinalis medialis and ( ) the teral aspects of the medulla oblongata, ros-tween the nucleus facialis and the nucleus vagus and caudally in the medial parts of ral reticular nucleus. A few APP and TH (PNMT) immunoreactive cells were also this area caudal to the area postrema, and m ial part of the locus coeruleus. APP TH NMT immunoreactive nerve terminals with ping distribution patterns were observed i.a. sympathetic lateral column of the spinal cord, tractus solitarii, locus coeruleus nucleus triangularis thalami, several hypothalamic (with particularly high concentrations the nical area. Results on the cat central nervous will not be reported here Absorption w th abolished the immunostaining in both pe al and central areas.

present findings give strong evidence that a lation of peripheral noradrenergic sympathetic ns innervating heart, vas deferens and other teral tissues contain an APP-like peptide is axonally transported This evidence is on (1) the identity of APP and TH immu-ctive cell bodies in sympathetic ganglia, ( ) distribution patterns for terminals and axons noreactive to these antisera, (3) disappear of APP and TH immunoreact e fibres in teral tissues after 6-OH DA treatment and (4) pearance of such fibres in the nasal mucosa sympathectomy.

the central nervous ystem the APP-l ke pep-was present in a population of adrenaline ral cell bodies belonging t the C1 and C? s of Hökfelt et al (1974) Furthermore over ng distribution patterns of APP and PNMT TH) immunoreactiv nerv terminals were in many areas of the brain and pinal cord (cf felt et al 1974) In addition APP was present in noradrenaline neurons of the locus coeruleus the A1 group of the lower medulla oblongata he present findings add a further example of coexistence of a peptide and a monoamine transm t ter in neurons (see Hökfelt et al 1980). The identity of the APP-like immunoreacti ity is not known and it will be an important task to elucidate its genuine structure The distribution of the APP-like peptide suggests a poss ble role i.a in the regulation of hemodynamic events

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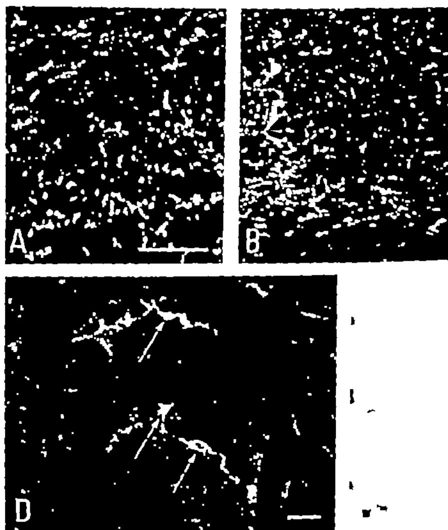
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Note added in proof Tatemoto and Mutt hav recently isolated PP-like peptide from mammalian intestine and brain (Nature 1980, 285 417)



F g 1A–E Immunofluorescence micrographs of the vas deferens (A–C) and ventrolateral medulla oblongata of untreated (A B) 6-hydroxydopamine (6-OH DA) (C) and colchicine (D E) treated rat after incubation to APP (A C D) and tyrosine hydroxylase (TH) (B E) D and E show the same section (elution according to et al 1978) (A–C) Dense network of APP immunoreact ve and catecholamine nerve terminals are seen in the deferens After 6-OH DA treatment most of the APP and TH (not shown) immunoreactive fibres disappear Neuronal cell bodies (arrows) conta both APP like immunoreactivity and TH Bars indicate 50 µm

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nfluence of circulating adrenaline on extracellular potassium concentration in the brain

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rculating adrenaline gains access to brain g by an abrupt elevation of arterial blood both cerebral blood flow (CBF) and he rate of oxygen (CMR_{O_2}) are markedly d (Berntman et al. 1978 Dahlgren et al. Probably this catecholamine effect is due to t effect on cerebral metabolism, with the in CBF following secondarily (cf. Mac et al 1976). However studies to date have nonstrated any reasonable link between the es in CBF and CMR_{O_2}, nor have they dis- the mechanism(s) causing the rise in CMR_{O_2}. hese studies have failed to reveal significant s in tissue concentrations of glycogen lac cyclic AMP or signs of neuronal activation ren et al 1980). Increases in CMR_{O_2} and CBF milar magnitude are found during seizures, then accompanied by an elevated concen- of extracellular potassium concentration for experimental results and further litera- ee Astrup et al 1979). It is therefore tempt speculate that adrenaline enhances CMR_{O_2} by ng K from cells and that this variable is also responsible for the increase in CBF

present study was undertaken to investigate ect of adrenaline on extracellular fluid [K] in rebral cortex. In order to eliminate the possi- of any direct effect on [K]e of the increase in pressure e have also included a series of l in which arterial pressure was increased in sence of added adrenaline

hod Male Wistar rats (300–450 g) were used besia was induced with 3% halothane and N_2O During operative procedures halothane educed to 1%. Following surgery the animals maintained on 70% N_2O and 30% O The ls were tracheotomized immobilized with urarine chloride (1.5 mg kg^{-1} i v) and con- d to a respirator In the adrenaline-infused s, femoral arterial and venous catheters were inserted In the other group, catheters were placed in the brachial arteries (see below). This allowed us to monitor blood pressure take samples for blood gas and pH analysis and to infuse drugs Following catheter insertion, the skull was exposed and a craniotomy was made on one side. The head was then immobilized and a double-barrelled K mic roelectrode (tip diameter 1–2 μm) was inserted through a small slit in the dura. The construction and use of the K electrode has been previously described (Astrup & Norberg 1976) The animal was then placed in a Faraday cage Rectal tempera- ture was maintained close to 37°C through the use of an infrared heating lamp All animals were main- tained under normoxic (PaO_2>100 mmHg) nor mocapnic ($PaCO_2$ 34–40 mmHg) conditions Two groups of animals were studied. In one group (n=18) adrenaline (8 μg kg^{-} min) was infused i v over a period of 10 min. In the other group (n=5) in order to induce a rise in arterial pres- sure the abdominal aorta was clamped and the clamp left in place over a period of 10 min. In both groups the K potential was continuously moni- tored before during and following either adrenaline infusion or aortic clamping. Statistical differences within groups were calculated using the paired Stu- dent's t-test.

R sults and discussion In both groups body temperature was kept close to 37°C. Blood gases remained at the levels mentioned above. Arterial blood pressure changes were strikingly similar be- tween the two groups (Fig. 1 upper part) This allowed us to distinguish any independent effect of an increased systemic blood pressure on brain ex tracellular fluid [K]

Fig. 1 (lower part) shows the changes in [K]e during 10 min of adrenaline infusion and 10 min of increased arterial pressure via clamping of the ab- dominal aorta. This figure clearly shows that a rise in arterial pressure, by itself has no effect on [K]e,

:aglomerular cell activity during hemorrhage ischemia as revealed by quinacrine ofluorescence

UND

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ÅLUND M. Juxtaglomerular cell activity during hemorrhage and ischemia as revealed by quinacrine histofluorescence. Acta Physiol Scand 1980. 110 113–121 Received 4 Dec. 1979 ISSN 0001–6772. Department of Histology Karolinska Institut and Department of General Surgery Karolinska Hospital, Stockholm, Sweden.

Quinacrine (QC) binds with high affinity to the intracellular storage granules of juxtaglomerular cells (JG-cells) in the afferent arterioles of the glomerulus of the kidney. The present study tests whether QC bound to JG-cells can be released. The cells were stimulated by renal ischemia and hemorrhagic shock combined with immobilization stress. 1 h after onset of renal ischemia QC JGI (modified Hartroft & Hartroft 1953) in ^{14}C-QC treated rats had decreased to about 40% in the ischemic kidney compared to not ligated control kidney. The ^{14}C-contents in the ischemic kidney had decreased to 33% of that in the untouched control kidney. Hemorrhagic shock was obtained by bleeding into reservoir for 15 min or 1 h. Rats who received QC or ^{14}C-QC 1 h before onset of bleeding showed no change in QC JGI (15 min shock) or ^{14}C-contents (1 h shock) as compared to controls. This was probably due to formation of new QC-binding granules which took up still circulating quinacrine thereby masking release. If the time between the QC injection and the onset of shock was extended to about 15 h when circulating amounts of QC are very low decrease of QC JGI (about 30% of controls) was seen in the kidneys of the shocked rats. The results are compatible with the possibility that QC in vivo bound to granules of JG-cells could be released together with the content of the granules following stimuli known to induce renin release. Quinacrine-binding therefore possibly provides new method to study endocrine cells in the way it has been used in the present study as marker of JG-cell activity

Key ords Quinacrine histofluorescence–juxtaglomerular cells–renal ischemia–hemorrhagic shock

acrine (QC) a fluorescent acridine derivative in selectively binds to nerve fibres and cell es in the gut and elsewhere (Olson et al. 1976, d 1978, Ålund & Olson 1978, 1979 b stock et al. 1978 b 1979 Olson & Ålund This binding seems to be closely linked to ous release mechanisms (Ålund & Olson 1978) QC bound to nerve fibres is released by ulation (Ålund & Olson 1978, 1979) Re ly it as reported (Ålund & Olson 1979 b c) that in n has a high affinity to hormone storing cles of endocrine cell containing dense core icles (Fig 1 b). Since such cells release hormones from their storage granules it was of interest to know whether QC presumably bound to the same vesicles (Fig. 1 *b*), could be released with the hormone or not.

Renin-release from the juxtaglomerular cells (JG-cells) of the afferent arteriolus of the glomer lus in the kidney has been thoroughly studied especially at renal hemodynamic disturbances. Although not endocrine cells in the classical sense JG-cells were chosen s a model for release activity Renal ischemia and hemorrhagic shock were used to induce release from the cells. The results indicate that QC bound to storage granules of the

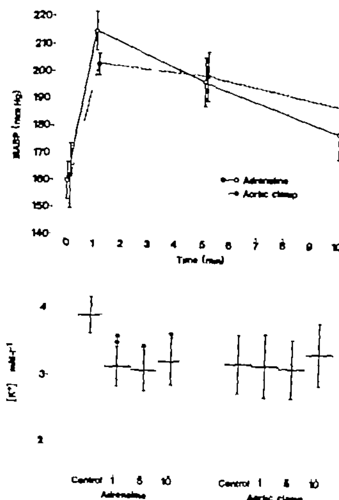


Fig 1 The influence of adrenaline and clamping of the abdominal aorta on arterial pressure (MABP) (upper part) and brain extracellular fluid [K] (lower part) over time Values are given as mean ± S E $P<0.001$ and $P<0.01$

However adrenaline causes a reduction in [K]e to about 80% of normal The reductions were all highly significant when comparing the [K]e at 1 ($P<0.001$) 5 ($P<0.001$) and 10 min ($P<0.01$) of adrenaline infusion to the [K]e prior to infusion

The results demon trate that the adrenaline induced increase in CMR cannot have been due to an elevation of [K]e e g due to an enhanced re lease from cells Rather it seems likely that the adrenaline induced reduction in [K]e was the result of an increased Na K pump activity (see Phillis 1974) Possibly the increase in CMR with adrena line is somehow related to this enhanced pump activity Yet the present result do not allow us to distinguish whether thi is a cause-effect relation ship or merely parallel phenomena However re sults from the present study indicate that extracel lular fluid [K] is not the link between the in creased CMR and increased CBF re enhanced tran location of adrenaline a blood-brain barrier at least not accor blassical concept of [K]e acting as a va Obviously the link between increased and blood flow still remains to be esta

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-f ontrol rats

.ung male rats were injected ith QC 1 mg/kg 1 h 15 h (3 rats) after QC as given their kidneys oved via the abdomen during ether anesthesia ared for QC-JGI estimation (see next paragraph)

ence microscopy and juxtaglomerular ion index

arious time-lapses and varied experimental pro- (see below) kidneys were rapidly dissected out e ether-anesthetized animal The kidney were along their lateral convex border freeze-dried Ungerstedt 1970) and paraffin-embedded.

araffin-embedded kidneys ere sectioned at 8 µm ay that the entire cortical area of the cut of the renal half was covered in one section. Sev blind coded sections were visually examined and ch represented the hole cut surface was selected rescence microscopy and estimation Specimens amined in dark-field fluorescence microscope d for Falck-Hillarp fluorescence histochemistry a & Jonsson 1967 Falck et al 1962) A light micro- l method to evaluate granule content in JG-cells cribed by Hartroft & Hartroft (1953) who used ecific granule stain according to Wilson (1952). ion of granule content in every single JG-ap- found in one section was made on semiquantita- ination scale with 4 steps (1–4) Step 1 represented aratuses ith cells containing few granules scat- ound the nucleus Step 2 meant one to three cells ranules spread throughout the cytoplasm but mpletely filling it Step 3 meant 1–3 cells with the asm filled ith densely packed granules or many the second category Step 4 meant several cells of d category The totals recorded under steps 1 2, 3 were multiplied by the factors 1 4 and 8 respec- ince, at very conservative estimate at least 8 s many granules were present in groups of cells ed as step 4, as ere present in those classified as (Hartroft & Hartroft 1953) Such eighed totals xpressed per 100 glomeruli to obtain juxtaglome- anulation index (JGI)

QC binds to and isualizes the storage-vesicles of -cells (Fig 1b Alund & Olson 1979) the same antitativ method to estimate granule content as was used in the fluorescence microscope to obtain rine juxtaglomerular granulation index (QC JGI). microscopy specimens were coded and all evalua- ere made blindly by the same investigator All aratuses on complete section from each kidney counted and evaluated

 formula for QC JGI as follows

$$I \quad \frac{b \qquad 8\,d}{N_{glom}} \; 100$$

 mber of JG-apparatuses (in the complete section) QC-fluorescent granule content estimated as step 1 mber of JG-apparatuses (in the complete section) QC-fluorescent granule content estimated as step mber of JG-apparatuses (in the complete section) QC fluorescent granule content estimated as step 3



Fig 2 ( ) Untouched kidney of rat h after single QC-injection of 1 mg/kg The cytoplasm of the juxta- glomerular cells (arrows) is densely packed with QC fluorescent granules. The glomerulus (glom) is not fluorescent. Tubular parts of the nephron are unspecifical- ly autofluorescent. Note that cell nuclei are not fluores- cent. Fluorescence microphotograph of 8 µm section, marker 50 µm. (*b*) The one hour ischemic kidney of the same rat as in (*a*). The cytoplasm of the juxtaglo- merular cells (arrow) in the afferent arteriole (aff) con- tains only few QC-positive granules. The glomerulus (glom) is nonfluorescent while tubular parts of the neph- ron are unspecifically auto-fluorescent Fluorescence microphotograph of 8 µm section, marker 50 µm

d = number of JG-apparatuses (in the complete section) with QC fluorescent granule content estimated as step 4 N_{glom} = total number of glomerulae found in the complete section.

Theoretically the QC JGI could vary within the limits 0 to 800 (800 when a, b and are 0 and d equal N_{glom}).

Quantitativ measurement of ¹⁴C-quinacrine ontent in kidneys

From the kidneys of animals tha had received ^{14}C-QC punches of about 10 mm³ were taken from cortical areas just beneath the surface of the kidney They were taken from the halves of the kidneys that were not used for fluorescence microscopy The punches were weighed (Cahn electrobalance), dissolved in soluene (Packard) and radioactivity measured by liquid scintillation (Packard Tri Carb 3002) Counts per minute per mg wet tissue were calculated ^{14}C-QC (spec. act. 16 mCi/mmol) was gener ous gift from Hoffmann la Roche Ltd Switzerland

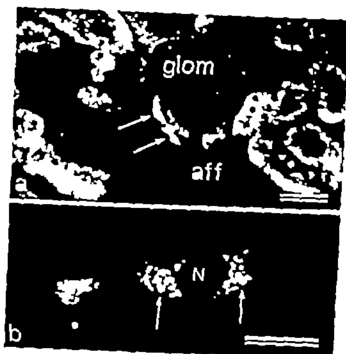


Fig 1 (*a*) Kidney of rat 1 h after QC i v 1 mg/kg Granules in the juxtaglomerular cells are intensely fluorescent (arrows) and therefore somewhat over exposed No fluorescence is found in the glomerulus (glom) or the afferent arteriolus (aff) Tubular parts of the nephron are unspecifically autofluorescent Fluorescence microphotograph of 8 μm section marker 50 μm (*b*) Close up on one JG-cell in the kidney of a rat that 1 h before sacrifice received QC 1 mg/kg i v The cytoplasm is filled with 25–30 intensely fluorescent densely packed granules (arrows) No fluorescence at all is found in the cell nucleus (N) To the left a minor part of a second JG-cell can be seen Fluorescence microphotograph of 6 μm section marker 10 μm

JG-cells (Fig 1) is released when reninrelease occurs QC can thus be used as a histofluorescent marker of juxtaglomerular cell activity

MATERIAL AND METHODS

56 male albino rats (Sprague Dawley 250 g b w) were used During a short period of ther anesthesia they were injected intravenously i the sublingual vein with quinacrine hydrochloride (sigma) or ¹⁴C labelled quinacrine hydrochloride (¹⁴C QC Hoffmann la Roche Switzerland) dissolved in saline The injected dose (QC as well a ¹⁴C-QC) was 1 mg/kg given in a volume of approximately 0.3 ml

Renal artery l gation

1 h after ¹⁴C-QC the right or left renal artery wa xposed (ether anesthesia) by a tran lumbar retroperitoneal approach and ligated in 8 rats. They were then allowed to wake up 1 h after ligation both kidneys were dissected out via an abdominal incision d ring ether-anesthesia. Punches for ¹⁴C-QC content meas rement were taken from both kidneys. The remaining part of t freeze-dried and paraffin-embedded. Not ligated were used as controls (untouched side)

Hemorrhagic shock

The 34 rats were given water ad libitum and ... diet which was withheld about 18 h before the ... or 1 –20 h before shock was started experimental ... received QC or ¹⁴C-QC i v The time between injection and shock will be referred to as ... below Every experimental animal with a QC ... and a subsequent bleed ng time of different leng... corresponding control animal with an equal leng... between QC injection and kidney dissection ... experimental design the 16 rats with longer QC-... 1 h did not all have a QC time of equal length ... experimental rats QC-time plus the bleeding ... kidney dissection ranged from 1 to 20 h (mean ... h) For the 7 control rats the time between QC ... and kidney dissection ranged from 12 to 20 h 15 6±0 9 h). The experimental procedure was ... according to Whigham & Well (1966) with ... tions (Farnebo & Hamberger 1977). The method ... established and will only be briefly described ... animals were anesthetized with ether during ... procedure (connecting animals to reservoir ... pressure transducer) Experimental rats were ... mobilized by being wrapped in metal nets (Corr... 1971) and allowed to wake up and recover ... before the onset of bleed ng They were bled ... voir to a systolic pressure of 70 mmHg measured ... a heparinized femoral artery catheter) by a ... transducer (Statham P23 AC) connected to a Gr... graph When they had had a stable low pressure ... or 1 h kidneys were dissected out (ether ... freeze-dried paraff n-embedded and sectioned ... (approximately 10 mm) were taken from cortical ... ¹⁴C-QC activity measurement Controls ... thetized by ether at an equal length of time as exper... rats In order to increase differences in renin ... trol were not shamoperated or immobilized.

C el m nation from pla ma after l C-QC

During ether anesthesia heparinized catheters ... serted in the right femoral artery of 4 rats ... for 8 h and heparin 300 U/kg (V trum Sweden) ... jected They were then given 1 mg/kg ¹⁴C-QC ... lingually and immobil zed by being wrapped ... net (Corrodi et al 1971) Blood samples (0 25 ml) were collected after 30 and 1 0 min (rats) or after and 40 min (rat) measured from the moment QC given i v After 4 h the metal nets (all 4 ...) femoral catheters were removed and animals ... leased 4 h after the QC njections blood samples collected from the left ventricle of the heart ... ples were immediately centrifuged (6000 g ... and 100 μl plasma was dissol ed in Soluene (Packard) counted by liq d scintillation. Using ¹⁴C toluene ... nal tandard the desintegrations per minute per ... plasma were calculated (dpm/100 μl pla ma).

ontrol rats

ng male rats were injected with QC 1 mg/kg. 1 h 15 h (3 rats) after QC was given their kidneys ved via the abdomen during ether anesthesia ed for QC JGI estimation (see next paragraph).

nce microscopy and ju taglomerular ndx

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raffin-embedded kidneys were sectioned at 8 µm way that the entire cortical area of the cut f the renal half as covered in one section. Sev- blind coded sections were visually examined and h represented the hole cut surface was selected scence microscopy and estimation. Specimens amined in dark-field fluorescence microscope for Falck Hillarp fluorescence histochemistry & Jonsson 1967. Falck et al. 1962). A light micro- method to evaluate granule content in JG-cells ribed by Hartroft & Hartroft (1953) ho used cific granule stain according to Wilson (1952). of granule content in every single JG-ap- ound in one section as made on semiquantita- ation scale ith 4 steps (1–4). Step 1 represented atuses ith cells containing few granules scat- und the nucleus. Step 2 meant one to three cells anules spread throughout the cytoplasm but pletely filling it. Step 3 meant 1–3 cells ith the in filled ith densely packed granules or many he second category. Step 4 meant several cells of category. The totals recorded under steps 1 2, 3 ere multiplied by the factors 1 2, 4 and 8 respec nce at ery conservative estimate, at least 8 many granules ere present in groups of cells d as step 4 ere present in those classified as Hartroft & Hartroft 1953). Such weighed totals essed per 100 glomeruli to obtain juxtaglome nulation ndex (JGI).

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$$\frac{b \quad \quad 8\,d}{N_{glom}} \; 100$$

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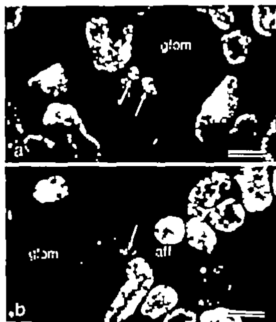


Fig. 2. ( ) Untouched kidney of rat 2 h after single QC-injection of 1 mg/kg. The cytoplasm of the juxta- glomerular cells (arrows) is densely packed with QC fluorescent granules. The glomerulus (glom) is not fluorescent. Tubular parts of the nephron are unspecifical- ly autofluorescent. Note that cell nuclei are not fluores- cent. Fluorescence microphotograph of 8 µm section, marker 50 µm. (b) The one hour ischemic kidney of the same rat as in (a). The cytoplasm of the juxtaglo- merular cells (arrow) in the afferent arteriolae (aff) con- tains only few QC-positive granules. The glomerulus (glom) is nonfluorescent while tubular parts of the neph- ron are unspecifically auto-fluorescent. Fluorescence microphotograph of 8 µm section, marker 50 µm.

d = number of JG-apparatuses (in the complete section) with QC fluorescent granule content estimated as step 4. N_{glom} = total number of glomerulae found in the complete section

Theoretically the QC JGI could vary within the limits 0 800 (800 hen a, b and c are 0 and d equals N_{glom})

Quantitati measurement of ¹⁴C-quinacrine content in kidney

From the kidneys of animals tha had received ¹⁴C-QC punches of about 10 mm³ were taken from cortical areas just beneath the surface of the kidney. They were taken from the halves of the kidneys that were not used for fluorescence microscopy. The punches were weighed (Cahn electrobalance), dissolved in soluene (Packard) and radioactivity measured by liquid scintillation (Packard Tri Carb 3002). Counts per minute per mg wet tissue were calculated. ¹⁴C-QC (spec act 16 mCi/mmol) was gener ous gift from Hoffmann la Roche Ltd, Switzerland

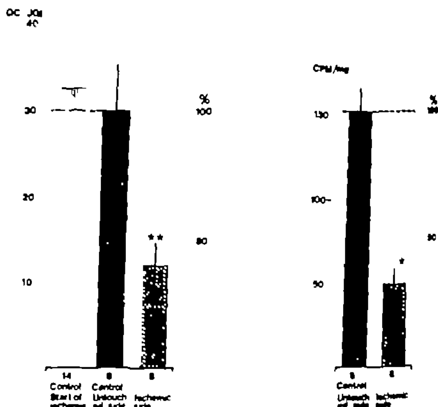


Fig 3 (*a*) Diagram to the left. Effects of renal ischemia (1 h bar to the right) on QC JGI values of animals which 1 h before ischemia received ^{14}C-QC 1 mg/kg i v A statistically significant ($P \leq 0.01$) reduction of QC JGI compared to the untouched kidney (middle bar) is found Both these bars represent the average of 4 right and 4 left kidneys The left bar represents QC JGI values found in 7 rats (7 right kidneys and 7 left kidneys, $n = 14$) 1 h after QC injection of 1 mg/kg (*b*) Diagram to the right Effects of 1 h renal ischemia on cpm/mg wet weight in animals which 1 h before ischemia received ^{14}C-QC 1 mg/kg i v A statistically significant ($P \leq 0.001$) decrease is seen on the ischemic side compared to the untouched side

Statistical analysis

For statistical analysis individual estimation were ranked and tested with the Mann-Whitney non-parametrical test Mean values ± S E M were used throughout this paper

RESULTS

Fluorescence features and QC JGI of controls

The 7 rats that were injected i v with 1 mg/kg QC showed a very intense QC fluorescence in the granules of the juxtaglomerular apparatuses in the afferent arteriolus 1 h later (Fig 1) In some JG cells most granules were located around the nucleus In others the entire cytoplasm was filled with densely packed granules If QC was given 1 –20 h before sacrifice the fluorescence was still quite strong but clearly weaker than after 1 h The QC fluorescence intensity in the granules was subjectively estimated to about 30 or 40% of the [illegible] after 1 h In addition to the QC fluorescence [illegible] is bright green the kidneys contained [illegible] autofluorescence This was located in the [illegible] parts of the nephron (Fig 1a a and b), [illegible] the proximal tubule It was first described [illegible] (I Sjöstrand thesis) The autofluorescence [illegible] green yellow or brown color and therefore [illegible] fluorescence microscope easily distinguishable [illegible] the bright green QC fluorescence QC JGI for those 7 control animals which had QC [illegible] h before sacrifice were found to be 3 6+3 4 left and right kidneys Fig 3a left bar) QC values for the 3 control animal that were sac[illegible] 15 h after a QC injection of 1 mg/kg were [illegible] be 5 1± .5 ($n = 6$ left and right kidney) [illegible] tematic difference between left and right [illegible] was found

ters ligation

escence in granules of the untouched kid- ned quite normal both concerning fluoresc- tensity and granule content (Fig. a). In the kidney granular fluorescence intensity unchanged but the granule content ap- to be reduced (Fig 2b) The untouched had QC JGI of 30.3±5 3 while the ischemic had decreased QC JGI of 12.1±2.6 or the control value (Fig 3) The difference ficant (P<0.01). The ischemic kidney con- only some 33 % of the ¹⁴C-counts/mg wet tis- od in the untouched kidneys (Fig. 3b). The e is significant (P<0.001) Separate values and right kidneys are given in Table 1

-lations between individual QC JGI and al ¹⁴C measurements were positive (r=0.70 The correlation between group mean QC JGI up mean ¹⁴C-QC contents given in Table 1 ongly positive (=0 92, n=4).

rh gic shock

orescence intensity of individual granules af morrhagic shock was not affected as com- to the control animals. The granules seemed normal and had a normal location within the In the experiment with long QC-time fluores- intensity had decreased as much in controls xperimental animals

ck 15 min QC-tim 1 h Bleeding volume in servoir at the end of the shock period ranged 8 to 3 2% of the body weight. QC JGI for experimental rats and the 3 control rats is in Fig 4 None of the QC JGI mean differ were significant

ck 1 h QC-time 1 h In this experiment ¹⁴C as used The average bleeding olume in the voir at the end of the shock period was 1 6 to of the body weight ¹⁴C contents of kidneys in

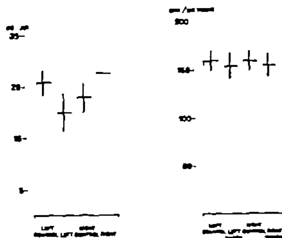


Fig 4 ( ) Diagram to the left. Bars representing QC JGI values of left and right kidneys of control animals and 15 min shock animals (1 h QC-time). No difference between shock and control group is revealed. (b) Diagram t the right kidneys of control animals and 1 h shock animals (1 h QC-time). No differences between shock and control group is revealed.

cpm/mg wet tissue are given in Fig. 4b No differ ence between experimental and control rats was found

Shock 1 h QC-time 12–20 h Average bleeding volume in the reservoir at the end of the shock period was 2.7 to 3 4% of the body weight. Both for

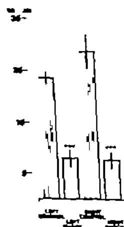


Fig 5 Effects on QC-JGI in right and left kidneys of animals with long QC-time (15.6 h on average). A statistically significant (P<0 001) of QC JGI is seen after 1 h of hemorrhagic shock.

1 Effect of chemia on quinacrine ontent taglomerular 8 Same animals as in fig 3

	Ischemic		Untouched	
	QC JGI	¹⁴C-QC	QC-JGI	¹⁴C-QC
ol				
kid-	7 8±. 9	53 18	39 3..5 7	179+ 9
(4)	14 5±3 1	45 8	21.3±6 6	125 16

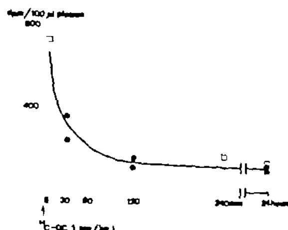


Fig 6 The elimination of ^{14}C from plasma in 4 rats (each symbol represents one rat) after injection of ^{14}C-QC 1 mg/kg Rats were immobilized from the ^{14}C-QC injection to minute 240 dpm = disintegrations per min

right and left kidneys a strongly significant reduction of QC JGI was found in the shocked animals ($P \leq 0.001$) (Fig 5) The decrease in granularity corresponds to 34 5% (left kidney) and 27 8% (right kidney) of corresponding control side

The difference between the left and right kidney was not significant

^{14}C-elimination from plasma after ^{14}C QC i v injection

4 rats were injected with ^{14}C-QC and elimination of ^{14}C from the plasma was studied The results are shown in Fig 6 Elimination of ^{14}C from plasma was initially rapid After 30–60 min about 50% of the activity at 5 min remains A steady state level seems to be reached after 120–240 min and thereafter the elimination over time occurs to be very slow

DISCUSSION

QC in vivo has a strong affinity to endocrine cells with large dense core vesicles (Ålund & Olson 1979 b) Since QC bound in vitro to nerve fibres in Auerbachs plexus (Olson et al 1976) can be released by depolarization in an at least partly Ca dependent manner (Ålund & Olson 1978 1979 a) it was interesting to see if QC in vivo bound to endocrine cells could be released by endocrine activity of the cells Although renin is an enzyme and not a hormone in the classical sense it is probably stored in the JG granules in a manner similar to th of polypeptide hormones in large dense co les For several reasons the JG-cells of the arteriolae in the kidney were chosen as a release activity First the rather large p these cells contain renin (Edelman & Hz and in vivo bind QC (Fig 1b Ålund be used for QC fluorescence microsc have devised a method to estimate gra the JG-cells which with slight modifica be used for QC fluorescence microsc providing a possibility to quantify QC in granules in these cells. The valid estimations is illustrated by the positive co between radioactivity and QC JGI in the ment with renal artery ligation Third and which trigger renin release in particul hemodynamic disturbances of the kidney known Ever since Goldblatt et al (1934 ered that renal artery constriction produ tained hypertension in the dog it has bee lated that renal ischemia is a primary st for renal renin release Convincing evidenc presence of an intrarenal vascular recept to renin release was provided by Blaine et a 1971 a b) Stimulation of renal nerves ha an increased renal renin release (Vand Johnson et al 1971) mediated via sympat tivity A β-receptor may trigger the incr nin-output (Winer et al 1969 Assayke 1970) Also circulating catecholamines renin secretion (Johnson Davis & Wri Chronic sodium depletion various humoral decreased sodium or chloride load at the densa also influence renin release (for Davis & Freeman 1976 and Reid et al 197

To obtain a strong patho-physiological for renin secretion severe bleeding comb immobilization stress was used In addition of the renal artery was used in some exp attempt to obtain maximal albeit non-ph cal release of JG-granules Both these probably influence JG-cells via one or sever above reviewed mechanisms

1 h after ligation of the renal artery the QC the ligated side decreased to about 40% (P the untouched control side The radioact punches after i v ^{14}C-QC in the ligated kid reduced to about 33% ($P \leq 0.001$) of (Fig 3a b) T [illegible] red gests that re [illegible] e

bound [14]C-QC has been released from the ...merular cell together with that granule ... Although most of the observed reduction ...tivity in the ischemic kidney probably re... release of QC from the JG-cells, some other ...must be considered. First, the ischemic kid... to the arterial ligation, probably contains ...ood than the untouched kidney. However the ... of blood that could be present in a volume ...m of kidney cortical tissue must be very ...and contribute only a few cpm. Th... be negligible amount compared to the total ...pm in the untouched kidney. Second, the ...ic kidney is not filtering. Therefore the pre... presence of urine in the punches might be ... the two situations. However the kidney ...mly ...ery small fractions of injected QC ...(1966, Thompson & Werbel 1972) and there...re presence or absence of small amounts of ...in primary urine in the punches probably ...butes ...ry little to the total amount of [14]C ...fore the major part of the difference ... ischemic and untouched kidney is prob...lue to release of granule content and [14]C-QC ... the JG-cells.

In hemorrhagic shock experiments with a QC time of 1 h and a shock period of 15 min or ...vealed no difference between control animals ...xperimental animals (Fig. 4 ... 6). One possible ...ination would be that no release has taken ... However since hemorrhage is known to in... renin-release and since a sharp reduction of ...[14]C and [14]C QC content is seen after ischemia ... h also is known to induce renin-release, this ...anation seem unlikely. Other factors might be ...due to consider. It has been described by sev... authors that the JG-cell is able to synthesize ...(... ate) renin very rapidly (Thurau et al. 1972, ...er et al. 1975). Such very rapid renin-activation ...been found to be induced by acute hemorrhage ... total renal ischemia (Weber et al. 1975). Since ...in is found exclusively inside the storage ...nules (Edelman & Hartroft 1961) a rapid in...se in renin also possibly imply that a rapid ...mation of new granules has occurred. If suffi...ntly concentrated QC ... present in the circula... hen hemorrhage induced the rapid formation ... new granules, the new granules would bind the ...ug. Thereby, even if a release of granular QC ...ntent, sufficient to cause a reduced QC JGI read... has taken place, it would be hidden by a simul...taneous increase of QC JGI due to formation of new QC-binding granules. This mechanism probably operates in the ischemic situation as well, with one important difference: the artery ligation makes it impossible for circulating QC to reach the implicated newly formed granules which theref we could not be "stained" by QC. The reduction of QC fluorescent granules will thus not be hidden in the same way as in the shock situation. Although the *amount of radioactivity* measured in plasma after a single injection of [14]C-QC is not only produced by circulating labelled QC but also by possible labelled metabolites of the drug, the diagram in Fig. 6 demonstrates that it is quite possible that a sufficient amount of QC is still circulating 1.2 h after an i.v. QC injection for QC binding to newly formed granules to occur. Moreover, hemorrhagic shock causes activation of several endocrine cell systems. Therefore a possible release of QC from e.g. adrenal medullary cells and other sources can increase the circulating level of QC.

The above interpretation of the data is supported when using a longer QC-time. Then QC JGI values in shocked animals were indeed found to be decreased ($p<0.001$, Fig. 5). This decrease indicates that a release of QC has taken place and that newly formed granules have not been marked by QC. Thus, with this technique the reduction of amounts of granules that were stained several hours before onset of bleeding is studied.

In conclusion, it is possible to show that acute renal ischemia, as well as acute hemorrhagic shock induces a decrease of QC JGI which most probably reflects a release of JG-cell granule content. Therefore it is possible that the recently reported in vivo QC binding to storage granules of certain endocrine cells (Ålund & Olson 1976*b*) can also be used to study endocrine cells in the way it has been used in the present study as a marker of JG-cell activity.

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e effect of dibutyryl cyclic AMP and PGE₂ on lysosomal zyme release and lactate production in relation bone resorption in vitro

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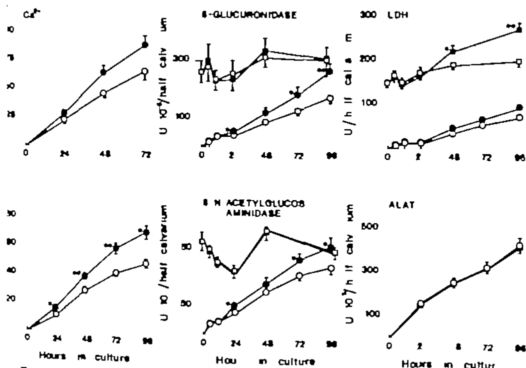
The effect of dibutyryl cyclic AMP (dbcAMP) and PGE₂ on the content and release of lysosomal and non-lysosomal enzymes was studied in bone organ culture system using half calvaria from 6–7-day-old mice. I parallel the effect of dbcAMP and PGE₂ on the release of calcium (Ca^{2+}) and inorganic phosphate (P_i) glucose consumption and lactate production was also followed DbcAMP (2.5 10^{-4} M) decreased the release of β-glucuronidase β-N-acetyl-glucosaminidase, acid phosphatase, Ca^{2} and P during the first day of culture During the 3rd and 4th day dbcAMP increased all these parameters. I contrast no changes in the release of lactate dehydrogenase (LDH) and alanine amino-transferase (ALAT) were seen Glucose consumption and lactate production was not stimulated by dbcAMP until the 3rd and 4th day On the other hand, PGE₂ (10^{-} M) stimulated the release of β-glucuronidase β-N-acetylglucosaminidase, Ca^{2+} and P as well as glucose consumption and lactate production already after 4 h and this stimulation was maintained throughout the culture period No effect by PGE₂ on the release of LDH and ALAT was registered The activities of LDH in the bone xplants after 96 h of culture were significantly augmented by both dbcAMP and PGE₂. It is concluded that bone resorption stimulated by dbcAMP and PGE₂, is associated with increased lysosomal enzyme release and lactate production

Key ords Bone resorption, lysosomal enzyme release, lactate PGE₂, cyclic AMP

clic AMP (cAMP) is considered to be an intracel-r mediator of the bone resorptive effect of athyroid hormone (PTH) and prostaglandins of E-type (PGE, PGE₂) (see review by Rasmussen Bordier 1974) We have studied the effect of MP on bone resorption in vitro and our results licate that cAMP in contrast to PTH PGE and E₂ stimulates bone resorption only after an in-l lag period or a period of reduced bone resorp- (Lerner & Gustafson, to be published) Thus consider cAMP to be a possible mediator only of delayed effect of PTH PGE and PGE₂ con-ivably related to the capacity of PTH and PGE₂ to cruit new osteoclasts. The cAMP induced inhibi-n of spontaneous mobilization of calcium and organic phosphate during the initial stages was und to be associated with a parallel reduction in lysosomal enzyme activities recovered from the lture media (Lerner & Gustafson 1979 Lerner 1979*a*). When PGE₂-stimulated bone resorption was inhibited by cAMP a parallel reduction of mineral mobilization and lysosomal enzyme release was also seen during the first 48 h (Lerner & Gustafson 1979*b*)

Our finding that cAMP has the capacity to inhibit lysosomal enzyme release agrees with the well-known inhibitory effect of cAMP on release processes of many other cells e g mast cells (Lichtenstei 1974 Orange et al. 1971) neutrophils (Ignarro 1973 Zurier et al. 1974) and macrophages (Weissmann et al 1971) but differs from two tudies which ha e been done on bone (Vaes 1968*a* Eilon & Raisz 1978).

In the experiments described in the present re port w have extended the culture period to 96 h and have examined the effect of dibutyryl cyclic AMP (dbcAMP) and PGE₂ upon both mineral and lysosomal enzyme release from bone calvaria. As



Kinetics of the effect of 10⁻ M PGE₂ on the release of calcium and inorganic phosphate activities of curonidase, β-N-acetylglucosaminidase, LDH and ALAT in media and bone plants from cultures of calvaral Groups of half calvaria (6-8 paired bones) were harvested after 0 6, 12 24 48 and 96 h and the enzyme activities assayed in media (circles) and bones (squares) In addition mineral analyses were performed in the media The ties of ALAT in the bones ere only determined after 96 h The control bones contained 105±14 U 10⁻⁴/half rium and the bones cultured in the presence of PGE 99_7 U 10⁻⁴/half calvarium Open ymbols represent values control cultures and filled symbols values from cultures exposed to PGE₂. Values are mean and SEM is given as a cal bar hen larger than the symbols height. P<0 05 **=P<0 01

ot in itself influence the release of ⁴⁵Ca from the

ULTS

t of dbcAMP nd PGE on the rel e nd P

n the cal arial bones were cultured in the pre e of 2.5 10⁻ M dbcAMP strong, significant bition of the release of Ca²⁺ and P was seen ng the first 4 h During the second day of ure no changes were registered but during the sequent two day significantly increased re e of both Ca² P was obtained (Fig. 1)
ddition of PGE₂ to culture media (10⁻ M) re ed in stimulation of the release of Ca²⁺ after 48 d P already after 4 h and this stimulation was ntained during the complete culture period (Fig. In other xperiments, not demonstrated in this er a significant release of both Ca² and P has been obtained after 24 h when PGE₂ (10⁻ M) was present in culture medium.

Effect of dbcAMP nd PGE₂ on th leas of lysosomal nzyme

The activity of β-glucuronidase in media from bones cultured in the presence of dbcAMP (5×10⁻⁴ M) was decreased during the initial 24 h but significantly enhanced during the following three days (Table 1). The bone explants exposed to dbcAMP (2.5×10⁻ M) for 96 h did not show any differences in the activity of β-glucuronidase as compared with untreated paired controls (Table 1) The activity in the total culture (bone after culture + medium) was significantly increased when the bones had been cultured in the presence of dbcAMP (Table 1)

When the activities of acid phosphatase and β-N-acetylglucosaminidase were assayed in the cal-



Fig 1 Kinetics of the effect of dbcAMP (2.5×10^{-4} M) on the spontaneous release of calcium (left) and phosphate (right) from calvarial bones cultured for 96 hours. Open columns represent values from control cultures, filled columns from cultures with dbcAMP Values are means ± S E for 7 paired expts

increased lactate production has been shown to be associated with PTH stimulated bone resorption (Vaes 1968*b*) glucose consumption and lactate production were also registered

MATERIALS AND METHODS

T ssue culture procedure

Calvarial (frontal and parietal) bones from 6–7-day-old mice (CsA type) were dissected aseptically and washed in Tyrode s solution Histological examination of sections of fixed and stained calvaria showed that they were free from blood and vascular cells and that the bones had a thin layer of periosteal cells covering the outer and inner sur faces. Care was taken under the dissection procedure not to damage the thin periosteum layer The calvaria were divided along the sagittal suture giving two halves one of which was used as a control bone and the other as an experimental bone The paired calvarial halves were then placed on separate stainless steel grids (Falcon 3014 Falcon Plastics Los Angeles USA) in multiwell culture dishes (Linbro Scientific Inc Hamden Conn USA) with ml of a modification of the chemically defined medium CMRL 1066 containing 0 1% albumin (w/v) Then dishes were put into plexiglass chambers and gassed with 5% CO in air The stationary cultures were maintained at 37°C for 96 h The media were changed every 4 h After culture the calvarial bones were placed in 0 2% (w/v) Triton X100 in saline for 1 h at 4°C and the supernatant used for enzyme assays. For further details, see Lerner & Gustafson 1979*a*

Quant fication of bone esorption

The magnitude of bone resorption wa assessed by follow ing the increase in concentration in the media of calcium (Ca) and inorganic phosphate (P) Ca was analysed according to Willis (1970) and P accord ng to Chen et al (1956)

Enzyme ass ys

The release of lysosomal enzymes from the bones was followed by registering the activities of β-glucuronidase β-N-acetyl-glucosam nidase and acid phosphatase in the media and the bones after culture The release of lysosomal enzymes was studied by determining tivities of lactate dehydrogenase (LDH) and a aminotransferase (ALAT) in the same samples as

β-glucuronidase (EC 3.2 1.31) was determined phenolphtalein-glucuronidate as substrate (Vaes & ues 1965) β-N-acetylglucosaminidase (EC 3 2 1 assayed with p-nitrophenyl N-acetyl-β-D-glucos as substrate (Vaes & Jacques 1965) and the liberated rophenol measured according to Seliinger et al Acid phosphatase (EC 3 1 3.2) was determined amount of p-nitrophenol liberated from p-nitro phosphate (Barret 1972) LDH (EC 1 1 1.27) was by determining the rate of oxidation of nicotinamide adenine dinucleotide at 340 nm (Wróblewski & LaDue 1955) ALAT (EC 2 6 1 assayed at 37°C according to Kabi Diagnostica (assays were performed under conditions where linear with respect to time and enzyme concentration unit refers to the decomposition 1 μmol substrate

Othe assays

Glucose consumption was estimated by determining decrease of glucose consumption in the media was determined by the coupled hexokinase phosphate dehydrogenase method (Barthelmai & 1962) Lactate in the media was analysed according Marbach & Weil (1967)

Material

Essentially fatty acid free bovine serum albumin V) Triton X100 phenolphtalein glucoronidate rophenyl N-acetyl-β-D-glucosaminide p-nitro phosphate pyruvate NADH hexokinase (yeast cose-6-phosphate dehydrogenase (yeast) NADP dehydrogenase (beef heart) NAD and N O adenosine 3 5 -cyclic monophosphate were from Sigma Chemical Co St Louis Mo USA alanine -oxo-glutarate and pyridoxalphosphate from Kabi Stockholm, Sweden PGE₂ (kindly supplied the Upjoh Co Kalamazoo MI through the court Dr Joh F Pike) was dissolved in ethanol The ethanol concentration in the experimental and control ture medium never exceeded 0 1% a concentrat

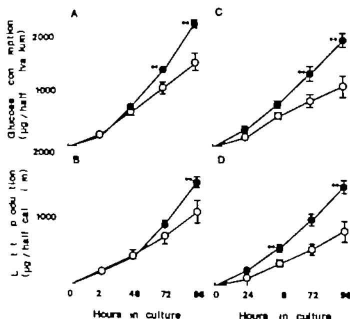


Fig 3 Kinetics of the effect of 5 10^{-4} M dbcAMP (A B) and 10⁻ M PGE₂ (C, D) on glucose consumption and lactate production in 96 hour cultures of calvarial bones. Open circles represent alues from control cultures and filled circles are from cultures treated ith dbcAMP or PGE₂. Values are means from 9 paired experiments and SEM is given as vertical bar hen larger than the symbol' height. $\cdot P<0.05$ $P<0.01$

of dbcAMP and PGE on the release -lysosomal enzymes

Table 2 it appears that the presence of MP in the culture media (2.5 10⁻ M) did not any changes in the release of LDH and [However both in the bones after 96 h of e and in the total culture an augmentation of mounts of LDH was registered while no dif es in the activities of ALAT were obtained

M PGE₂ did not produce any changes in the e of LDH and ALAT from the bones to the e media (besides small significant increase H after 96 h) However in the bones, a stimu- of the activity of LDH was seen after 48 h and In contrast, no changes of ALAT was regis- in the explants (Fig).

of dbcAMP nd PGE on glucos mption and lact i production

s be seen in Fig. 3 both glucose consumption actate production was unaltered during the o day when the bones were cultured in the nce of dbcAMP (2.5 10⁻ M) During the sub- nt tw days bones exposed to dbcAMP consumed more glucose and produced more lactate than untreated bones.

Addition of PGE₂ to culture media (10⁻ M) resulted in increased glucose consumption and lactate production during all the four days (Fig. 3)

DISCUSSION

Lysosomal enzymes nd bone resorption

Our finding that bone resorption promoted by PGE₂ is associated with an increased release of lysosomal enzymes is in agreement with recent observations by Eilon & Raisz (1978). The additional finding that dbcAMP enhanced the release of three lysosomal enzymes in parallel with stimulation of bone resorption confirms the results reported by Vaes (1968a) Eilon & Raisz (1978) and Lerner & Gustafson (1979c). However in contrast to PGE₂ dbcAMP caused an initial reduction of lysosomal enzyme release and mineral mobilization an observation which agrees with earlier results from this laboratory (Lerner & Gustafson 1979) The effects of PGE₂ and dbcAMP on enzymes from lysosomal stores are specific, since the release of the non-

Table 1 *The effect of dbcAMP (5×10 M) on the content and release of lys somal en calvarial bones maintained in organ culture for 96 h*

Values are means±SE for 7–9 paired expts

	Medium					
	0– 4 h	4–48 h	48–7 h	7?–96 h	Bone after 96 h	Total culture
β-glucuronidase (U×10⁻⁴/half calvarium)						
Control	3 9± 8	5 7±3 0	?4 4± 9	17 6± 0	161±16	34
dbcAMP	2 2± 3	49 3±5 7	51 0±4 8*	42.0±6 4	195±32	3??
Acid phosphatase (U×10⁻ /half calvarium)						
Control	29 0±5 0	18 0±2 6	19 5±1 6	15 8±2 7	1 617±29	1??
dbcAMP	14 4±4 3	26 4± 5	49.5±7 4	35 4±3 4	2 576±284	2?
β-N-acetylglucosaminidase (U×10⁻⁴/half calvarium)						
Control	61.8±4 3	31 7±2 4	44.3±3 1	37 1±3 4	190±18	??
dbcAMP	?1 8±0 7	?6 6±1 3	57 2±3 6	40 2±6 6	14±16	??

* $P<0.05$ * $P<0.01$ * $P<0.001$

Bone after culture + media

ture media the kinetic pattern was similar to that seen when *β* glucuronidase was registered Thus the presence of dbcAMP (? 5×10 M) in the culture media resulted in a strong inhibition during the first 24 h During the second day no striking differences were discernible but during the third and fourth day the activities in the media from bones exposed to dbcAMP were enhanced although the effect as regards to the activities of *β* N acetylglucosaminidase was not too pronounced (Table 1) The amounts of acid phosphatase in the bones after 96 h of culture were significantly increased after addition of dbcAMP to the culture media as were the activities of acid phosphatase in the total culture (Table 1)

When the bone explants were treated with PGE₁ a stimulation of the release of *β* curonidase and *β* N-acetylglucosaminidase registered after 24 h and this stimulation was tained for 96 h (Fig ?) No differences in the tivities of *β*-glucoronidase and *β*-N-acetyl cosaminidase between PGE₁-treated and co bones were registered during the culture p (Fig ?) It can also be seen from Fig that control and experimental bones released amounts of the two acid hydrolases Despite there were no changes in the activities in the after 96 h as compared with bones from da both as regards control and experimental bone

Table 2 *The effect of dbcAMP (2.5×10 M) on the content and release of LDH and ALAT from bones maintained in organ culture for 96 h*

Values are means ± S E for 9 paired expt

	Medium					
	0– 4 h	4–48 h	48–72 h	72 96 h	Bone after culture	Total culture
LDH (U/half calvarium)						
Control	?9 6±1 9	3 7± 6	5.3± 1	4 4± 8	?89±12	399 1?
dbcAMP	3 9± 4	30 4±2	?9 3± 8	8 2± 7	37?±?0*	4?? 1?
ALAT (U×10⁻ /half calvarium)						
Control	134±9	143±11	116±5	109±13	163±15	665 ??
dbcAMP	137±6	147±1	104±11	87±1	187±13	661 1

* $P<0.001$

Bone after culture + media.

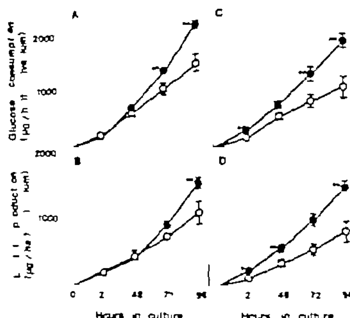


Fig. 3. Kinetics of the effect of 2.5 × 10 M dbcAMP (A, B) and 10 M PGE$_2$ (C, D) on glucose consumption and lactate production in 96 hour cultures of calvarial bones. Open circles represent values from control cultures and filled circles are from cultures treated with dbcAMP or PGE$_2$. Values are means from 9 paired experiments and SEM given as a vertical bar when larger than the symbol height. * P<0.05 ** P<0.01

of dbcAMP and PGE on the release of lysosomal enzymes

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M PGE did not produce any changes in the of LDH and ALAT from the bones to the e media (besides a small significant increase H after 96 h). However in the bones stimu- of the activity of LDH was seen after 48 h and In contrast, no changes of ALAT was regis- in the explants (Fig.)

of dbcAMP and PGE$_2$ on glucose mption and lactate production

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Addition of PGE to culture media (10 M) resulted in increased glucose consumption and lactate production during all the four days (Fig. 3)

DISCUSSION

Lysosomal enzymes and bone resorption

Our finding that bone resorption promoted by PGE$_2$ is associated with an increased release of lysosomal enzymes is in agreement with recent observations by Eilon & Raisz (1978). The additional finding that dbcAMP enhanced the release of three lysosomal enzymes in parallel with stimulation of bone resorption confirms the results reported by Vaes (1968a), Eilon & Raisz (1978) and Lerner & Gustafson (1979c). However in contrast to PGE$_2$ dbcAMP caused an initial reduction of lysosomal enzyme release and mineral mobilization an observation which agrees with earlier results from this laboratory (Lerner & Gustafson 1979a). The effect of PGE$_2$ and dbcAMP on enzymes from lysosomal stores are specific, since the release of the non-

Table 1 *The effect of dbcAMP (2.5×10 ⁴ M) on the content and rele se of lys somal enz calvarial bones maintained in organ culture for 96 h*

Values are means±SE for 7–9 paired expts

	Medium					
	0–24 h	4–48 h	48–72 h	7? 96 h	Bone after 96 h	Total culture
β-glucuronidase (U×10⁻⁴/half calvarium)						
Control	3 9± 8	25 7±3 0	4 4±2 9	17 6± 0	161±16	3[illegible]
dbcAMP	2.2± 3	49.3±5 7	51 0±4 8	4 0±6 4	195±32	39[illegible]
Acid phosphatase (U×10⁻ /half calvarium)						
Control	79 0±5 0	18 0± 6	19.5±1 6	15 8±.7	1 617±229	16[illegible]
dbcAMP	14 4±4 3	26 4± .5	49.5±7 4	35 4±3 4	2 576±284*	2[illegible]
β-N acetylglucosaminidase (U×10⁻⁴/half calvarium)						
Control	61 8±4 3	31 7±2 4	44.3±3 1	37 1±3 4	190±18	36[illegible]
dbcAMP	21 8±0 7*	26 6±1 3	57 2±3 6	40.±6 6	214±16	36[illegible]

* = $P<0.05$ ** = $P<0.01$ *** = $P<0.001$
Bone after culture + media

ture media the kinetic pattern was similar to that seen when β glucuronidase was registered Thus the presence of dbcAMP (2 5×10 M) in the culture media resulted in a strong inhibition during the first 24 h During the second day no striking differences were discernible but during the third and fourth day the activities in the media from bones exposed to dbcAMP were enhanced although the effect as regards to the activities of β-N acetylglucosaminidase was not too pronounced (Table 1) The amounts of acid phosphatase in the bones after 96 h of culture were significantly increased after addition of dbcAMP to the culture media as were the activities of acid phosphatase in the total culture (Table 1)

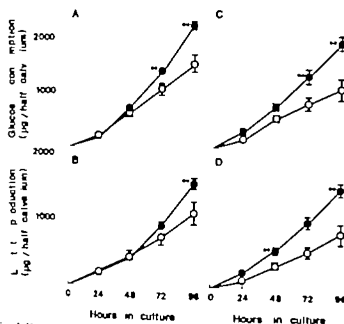
When the bone explants were treated with [illegible] PGE_2 a stimulation of the release of [illegible] curonidase and β-N-acetylglucosaminidase registered after 24 h and this stimulation was [illegible] tained for 96 h (Fig 2) No differences in the [illegible] tivities of β-glucoronidase and β-N-acet[illegible] cosaminidase between PGE_2-treated and co[illegible] bones were registered during the culture p[illegible] (Fig 2) It can also be seen from Fig. 2 that control and experimental bones released [illegible] amounts of the two acid hydrolases Despite there were no changes in the activities in the b[illegible] after 96 h as compared with bones from da[illegible] both as regards control and experimental bone[illegible]

Table 2 *The effect of dbcAMP (2 5×10 M) on the content and release of LDH and ALAT from cal bones maintained in organ culture for 96 h*

Values are means ± S E for 9 paired expts

	Medium					
	0–24 h	24–48 h	48–72 h	72 96 h	Bone after culture	Total culture
LDH (U/half calvarium)						
Control	29 6±1 9	3 7± 6	25 3±2 1	4 4± 8	289_1	399 13
dbcAMP	3. 9± 4	30 4±2	29 3± .8	28.±2 7	372±20*	494 [illegible]
ALAT (U×10⁻³/half calvarium)						
Control	134±9	143±11	116±5	109±13	163±15	665±13
dbcAMP	137±6	147±12	104±11	87±12	187±13	663 [illegible]

* = $P<0.001$
Bone after culture + media



F g 3 Kinetics of the effect of 5 10⁻⁴ M dbcAMP (A, B) and 10 M PGE₂ (C D) on glucose consumption and lactate production in 96 hour cultures of calvarial bones. Open circles represent alues from control cultures and filled rcles are from cultures treated th dbcAMP or PGE₂. Values are means from 9 paired periments and SEM given as ertical bar hen larger than the ymbol' height $*P<0.05$ $P<0.01$

of dbcAMP nd PGE on the release lysosomal enzymes

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Addition of PGE₂ to culture media (10⁻ M) resulted in increased glucose consumption and lactate production during all the four days (Fig 3)

DISCUSSION

L sosomal enzymes nd bone resorption

Our finding that bone resorption promoted by PGE₂ is associated with an increased release of lysosomal enzymes is in agreement with recent observations by Eilon & Raisz (1978) The additional finding that dbcAMP enhanced the release of three lysosomal enzymes in parallel with stimulation of bone resorption confirms the results reported by Vaes (1968a) Eilon & Raisz (1978) and Lerner & Gustafson (1979) However in contra t to PGE₂, dbcAMP caused an initial reduction of lysosomal enzyme release and mineral mobilization an observation which agrees with earlier results from this laboratory (Lerner & Gustafson 1979) The effects of PGE₂ and dbcAMP on enzymes from lysosomal stores are specific, ince the release of the non-

Table 1 *The effect of dbcAMP (2.5×10 ⁻³ M) on the content and release of lysosomal enzym calvarial bones maintained in organ culture for 96 h*

Values are means±SE for 7–9 paired expts

	Medium				Bone after 96 h	Total culture
	0–24 h	4–48 h	48–72 h	7? 96 h		
β-glucuronidase (U×10 /half calvarium)						
Control	3 9±2 8	‿5 7±3 0	?4 4±2 9	17 6±‿0	161±16	3‿
dbcAMP	22 2± .3	49 3±5 7	51 0±4 8	42.0±6.4	195±3‿	3?
Acid phosphatase (U×10 /half calvarium)						
Control	29 0±5 0	18 0±? 6	19 5±1 6	15 8± 7	1 617+‿29	1 ‿
dbcAMP	14 4±4 3	?6 4±2 5	49 5±7 4	35 4±3 4	576±2‿	?
β-N-acetylglucosaminidase (U×10⁻⁴/half calvarium)						
Control	61 8±4.3	31 7± 4	44 3±3 1	37 1±3 4	190+18	?
dbcAMP	?1 8±0 7*	?6 6±1 3	57.‿±3 6	40 ±6 6	14±16	?

* = $P<0.05$ = $P<0.01$ = $P<0.001$

Bone after culture + medm

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Values are mean ± S E for 9 paired expts

	Medium				Bone after culture	Total culture
	0–24 h	4–48 h	48–72 h	72 96 h		
LDH (U/half calvarium)						
Control	?9 6±1 9	32 7± 6	‿5 3±2 1	24 4±2 8	‿89±1	39? 1?
dbcAMP	3 9± 4	30 4± 2	29 3±2 8	28 ±2 7	372±?0	49? 1?
ALAT (U×10 /half calvarium)						
Control	134±9	143±11	116±5	109±13	163±15	66? 1?
dbcAMP	137±6	147±1	104±11	87±1	187±13	663 1

* = $P<0.001$

Bone after culture + media

...inal cells. Alternati ely the augmentation of ... could be a consequence of an increased ...r of cells. It should, however be pointed out ...e effect of dbcAMP and PGE_2 seems to be ...ve to that degree that the activities of ALAT ...unchanged

...ork was supported by grant from the S edish ...ation against Rheumatic Diseases, from the Royal Fund of King Gustav V and from the Swedish ...l Research Council (project No B79-14X-05426-

...author gratefully acknowledge M Anita Johans... Ingrid Karlsson and Mrs Britta Lindgren for ...ful assistance Ms Birgitta Wiklund and Mrs ...a Wrethen for typing the manuscript, Mrs Y onne ...son for drawing the illustrations and Dr Ian Jones ...ntic improvements of the manuscript

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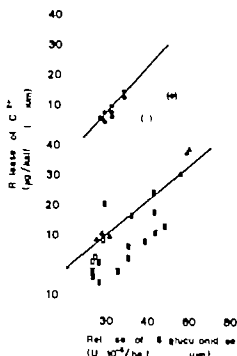


Fig 4 Correlation between the release of β-glucuronidase and bone resorption evaluated by the release of calcium from cultured calvarial bones. The explants were cultured for 96 hours either without (open symbols) or with 5×10⁻ M dbcAMP (filled symbols). Data in the upper figure are from the initial 24 h of culture (=0 85 $P<0\ 001$) Data in the lower figure are from the 3rd (△ ▲) and 4th (□ ■) day of culture (=0 82 $P<0\ 001$)

lysosomal enzymes LDH and ALAT was uninfluenced As demonstrated in Fig 4 there is a significant correlation between bone resorption estimated as the release of Ca and release of β-glucuronidase both as regards the inhibitory and stimulatory effect of dbcAMP These data support the concept of Raisz (1976) that although the specific roles for several lysosomal enzymes released during bone resorption has not been defined the breakdown of bone matrix material probably requires such enzyme activities With regard to the degradation of collagen which is the major organic component the current opinion seems to be that in most tissues the initial stage involves an extracellular degradation by means of neutral collagenases (Harri & Cartwright 1977) However in contrast there is some controversy about the further breakdown whether it is a mainly extra or intracellular process One proposal is that after the primary collagenolysis the second attack is done either by extracellular neutral proteinase or by intracellular lysosomal enzymes (Harri & Cartwright 1977) Another opinion i that the lysosomal enzymes are released to the extracellular milieu and the[...] tribute to the breakdown of collagen (Va[...] Although there seems to be an intimate [...] between bone resorption and lysosomal e[...] lease data from the present and earlier [...] tions (Vaes 1968*a* Vaes 1968*b* Eilon & [...] Lucht 1971 Doty & Schofield 197) do not [...] conclusive evidence that the acid hydrolas[...] their effects extracellularly However the [...] ous invaginations of the osteoclast ruffled [...] could very well offer an extracellular en[...] ronment suitable for the acid lysosomal en[...] analogy with the conditions associated w[...] age matrix degradation as suggested by [...] (1978 1979)

Glucose consumption lactate production and bone resorption

Bone resorption either promoted by PG[...] shown in the present paper or promoted b[...] (Vaes 1968*b*) seems to be associated [...] creased lactate production The present pa[...] shows that while dbcAMP initially decrea[...] resorption without affecting glucose cons[...] and lactate production the delayed bone re[...] effect of this cyclic nucleotide was also as[...] with increased glucose consumption and l[...] production As both 1,25 $(OH)_2D_3$ (Bro[...] Neuman 1979) and 1α(OH)D (Lerner 19[...] been shown to stimulate bone resorption [...] increasing glucose consumption and lactate [...] tion it seems that agents which increase b[...] sorption and the level of cAMP (PTH [...] dbcAMP) concomitantly increase the prod[...] lactate while hydroxylated derivatives of [...] D which do not increase cAMP promot[...] resorption without any change in lactate [...] tion It is therefore possible that increased [...] production may contribute to bone resorp[...] that it is obviously not an indispensable pa[...] mechanism

Our finding in the present report that st[...] of bone resorption by PGE and dbcAMP [...] sociated with increased content of LDH [...] bones does not nece sarily implicate a role [...] in bone resorption It is interesting howev[...] LDH by means of ultrastructural hi toche[...] has been shown to be concentrated in cyto[...] vacuoles near the ruffled border in the oste[...] (Coleman et al 1976) Thus PGF_2 and db[...] may have increased the content of LDH

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protein lipase of human postheparin plasma and adipose ie in relation to physical training

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MARNIEMI J PELTONEN P VUORI I & HIETANEN E.. Lipoprotein lipase of human postheparin plasma and adipose tissue in relation to physical training Acta Physiol Scand 1980, 110: 131 135 Received 28 Dec. 1979 ISSN 0001-6772. The Rehabilitation Research Centre of the Social Insurance Institution, Turku and Department of Physiology University of Turku, Finland.

Adipose tissue lipoprotein lipase and postheparin plasma triglyceride lipase activities were measured in 28 men differing in their physical training activity They were divided into 4 subclasses based on their training intensity The two most active classes (17 subjects) having regular heavy exercise at least 4 times week were considered as the actively training group and the other two (11 subjects) classes not training regularly the control group. In postheparin plasma, the lipoprotein lipase activities were not different between the two groups, whereas training subjects had significantly ($P<0.02$) lower hepatic lipase activities. Adipose tissue lipoprotein lipase activity was in the training group at about 70% higher level on an average than in the control ($P<0.10$). A significant positive correlation ($r=0.38$, $P<0.05$) was obtained between the adipose tissue lipoprotein lipase activity and the level of physical activity Our data suggest that even moderate inter-group differences in the physical training activity are reflected as measurable alterations in the adipose tissue lipoprotein lipase activity in man

Key words Lipoprotein lipase hepatic lipase postheparin plasma, adipose tissue physical training, man

otein lipase being located in the capillary elium of adipose and muscle tissue is a key e in the catabolism of chylomicrons and very ensity lipoproteins (Nikkilä 1953). These atic activities and furthermore l pase of origin are known to be released into the tion by heparin injection both in laboratory and in man (Robinson 1970) These lipoly ymes have been recently studied especially sity (e g. Persson 1973 Campbell et al 1974 en et al. 1975 Pykälistö et al 1975 Taskinen Nikkilä 1977 Schwartz & Brunzell 1978) and tion familial hypertriglyceridemias (Persson Huttunen et al 1976).

role of physical exercise as a possible deter t of lipoprotein lipase has gained less atten- In laboratory animals physical training rstapa et al. 1975 Parizkova 1977) and short physical exercise (Nikkila et al. 1963 and 1965 et al. 1977) have been shown to increase the lipoprotein lipase activity of the skeletal and heart muscle although also contradictory results have been reported (Askew et al 1977).

Recently this problem has been investigated also in man in some studies. In champion class long distance runners lipoprotein lipase activity appeared to be considerably higher than in sprinters or control subjects (Nikkilä et al. 1978b) I our previous study no immediate response of postheparin plasma lipoprotein lipase to short-term (1 h) exercise was found (Marniemi et al 1978) which is in accordance with the negligible changes obtained in skeletal muscle lipoprotein lipase activities after the same type of short exercise (Lithell et al 1979) However in the connection of these short term studies we noticed a tendency towards higher lipoprotein lipase values in well-trained subjects (not published) which encouraged us to carry out the current study

The purpose of the present study was to investi-

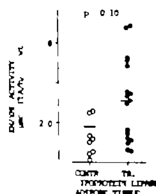
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Adipose tissue lipoprotein lipase activities in con- -pen circles) and trained subject (black circles). ...er explanations, see legend in Fig. 1 and Method

...ean of lipoprotein lipase activities of the ...l subjects was about 70% higher than in the ...ls (Fig) Due to the rather marked inter in- ...al variation, the statistical significance of the ...nce was low ($P<0.10$) However only in one ...t of the control group the enzyme activity ...igher than the mean of the training group ...)

...re was tendency to a positive correlation ...44 $P>0.10$) between adipose tissue and post ...in plasma lipoprotein lipase activities in the ...ol group Calculated from the training or all ...ts the correlation was even poorer Hepatic activity did not correlate significantly with ...ther lipolytic activities measured

...pose tissue lipoprotein lipase activity ap- ...d to be related to the degree of the training ...ity A significant positive correlation ($r=0.38$ ...05) was found to exist between the enzyme ...y and the degree of physical activity expres- ... the four training subclasses (Fig 3).

...USSION

...ding to the present preliminary data the en- ...ng effect of physical training on adipose tissue ...otein lipase in man is suggested. This accord ...the recent results of Nikkilä et al (1978b) ...sed with champion class long distance runners ...printers. Because of the marked inter ind ...l variation, statistically only a tendency to- ...higher values was found in the present study ...he results have to be confirmed by studying ...ffect of a controlled training program in previously untrained subjects. These studies are in our next research program.

In postheparin plasma the relation between lipoprotein lipase activity and the level of physical training was not demonstrable However lipoprotein lipase activity in postheparin plasma originates from several tissue pool such as adipose tissue and skeletal muscle (Robinson 1970) Accordingly a very poor correlation was obtained between post heparin plasma and adipose tissue lipoprotein lipase activities, which is in agreement with earlier reports (Guy-Grand & Bigorie 1975 Taskinen & Nikkilä 1977). All these data stress the preference of using tissue biopsies rather than postheparin plasma in evaluating lipoprotein lipase activity in vivo

The differences in adipose tissue lipoprotein lipase activities between the groups cannot be explained by different relative weights of the subjects, because this enzyme acti ity is known to be independent on the degree of obesity when calculated on weight unit basis (Pykälistö et al. 1975 Taskinen & Nikkilä 1977 Lithell et al. 1978)

It is of interest in this connection that a positive correlation between adipose tissue lipoprotein lipase activity and plasma HDL-cholesterol concentration has been reported in recent studies of Nikkilä's group (Nikkilä et al. 1978a and 1978b Taskinen & Nikkilä 1979) In addition, persons with high physical acti ity at work (Lehtonen & Viikari 1978) or in leisure time (e.g. Carlson & Mossfeldt 1964 Wood et al. 1976) are known to ha e elevated HDL-cholesterol levels in the circulation and high

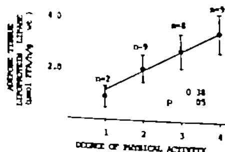


Fig 3 Correlation between adipose tissue lipoprotein lipase activities and the degree of physical activity expressed as the training class. Indicated are the means of the enzyme activities and their standard errors and the number of subjects (n) in each class. The correlation coefficient ( ) and the equation of the curve were calculated by linear regression analysis. For more details about the training classes, see Methods.

Table I *Ages and body characteristics of actively training and non-training control subjects*
Values are indicated as means ± S E. The relative body weight was calculated from Geigy Tables. For the classification of the groups see Materials and Methods

	n	Age (yr)	Height (cm)	Weight (kg)	Relative body weight (%)
Controls	11	29 0±1.2	178 0± 0	78.5±2.9	103.6±3.9
Training	17	33.5±1 4	179±1.3	72.4±_1	92.7±2.0

gate the role of physical fitness in lipoprotein lipase activity by measuring the enzyme activity in regularly training subjects differing in the intensity of the training This was accomplished in order to find out if small differences in the physical activity are reflected in lipolytic activity levels

MATERIALS AND METHODS

Test subjects 28 healthy men without having any known disorders of fat metabolism were given a detailed questionnaire about their physical training activity Based on their answers the subjects were divided into 4 subgroups The groups were as follows. the most active subclass (number 4) was training or competing in heavy physical exercises like running swimming cross-country skiing soccer etc regularly at least 4 times a week for the last 12 months or more the next subgroup (number 3) was having these exercises at least 3 h a week the following class (number 2) was having light exercise like walking garden work etc at least 4 h a week and the last subclass (number 1) consisted of subjects having clearly less physical exercise than the preceding group The two most active subclasses were regarded as the actively training group (17 men) and the two less active classes as the non-training control group (11 men). The mean ages and body characteristics of the test subjects in both groups are shown in Table 1 In spite of the higher relative weight in the control group only two subjects in this group could be regarded as obese (relative weight >110% accord ng to Geigy Tables)

Sample and chemical analys Postheparin plasma was obtained from the antecubital vein of sitting subjects 10 min after an i v injection of heparin (50 IU/kg) This heparin dose is optimal for the release of hepatic lipase (Huttunen et al 1975*b*) and compared with clearly higher and more risky doses (100–200 IU/kg) it gives a fairly representati e release (70–80%) of the endothelially bound lipoprotein lipase (Krauss et al 1974 Sauar et al 1978). The samples were immediately cooled centrifuged at +4°C and stored at −70°C at most one month until analyzed Lipoprotein lipase and hepatic lipase activities were measured by the specific immunochemical method of Huttunen et al (1975*b*) Specific antiserum against hepatic lipase was a generous gift from Dr Christian Ehnholm Helsinki, Finland Adipose tissue specimens about 100 mg wet weight) were taken as needle (i.d. diameter 2 mm) biopsies from the abdominal region into physiological saline solution and stored intact at 20°C months before enzyme analyses. Their lipoprotein lipase activities were determined according to the method of Hietanen & Greenwood (1977).

The statistical significances of the differences between the groups were assessed by the two-tailed Student's *t*-test.

RESULTS

The individual activities of postheparin *lipoprotein lipase* and hepatic lipase are presented in Fig 1 Lipoprotein lipase activities of the trained subjects were not significantly different from those of the control group On the other hand hepatic lipase activity of postheparin plasma was significantly higher (46% on an average $p<0.05$) in the trained controls than in the actively trained subjects (Fig 1) In the adipose tissue instead

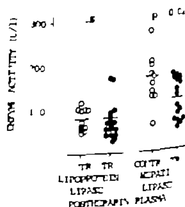
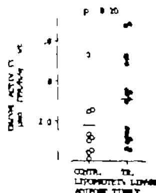


Fig 1 Postheparin plasma lipoprotein lipase and hepatic lipase activities in controls (open circles) and trained subjects (black circles) The means of the activities of each group are indicated by horizontal lines. Enzyme activities are e pressed units/litre (U/l), where one unit is defined as one µmole fatty acid liberated/minute The significances of the differences in enzyme activities between the two groups are also given (N.S = not significant). For detail see Methods.



Adipose tissue lipoprotein lipase activities in con- pen circles) and trained subject (black circles). er explanations, see legend to Fig. 1 and Methods.

ean of lipoprotein lipase activities of the subjects was about 70% higher than in the (Fig) Due to the rather marked inter-in- al variation, the statistical significance of the nce was low ($P<0.10$). However only in one t of the control group the enzyme activity igher than the mean of the training group)

re was a tendency to positive correlation 14 $P>0.10$) between adipose tissue and post n plasma lipoprotein lipase activities in the al group. Calculated from the training or all ts the correlation was even poorer Hepatic activity did not correlate significantly with her lipolytic activities measured

pose tissue lipoprotein lipase activity ap- f to be related to the degree of the training ty A significant posit ve correlation (=0.38, 05) as found to exist between the enzyme y and the degree of physical activity expres the four training subclasses (Fig 3)

USSION

ding to the present preliminary data the en- ng effect of physical training on adipose tissue otein lipase in man is suggested. This accords the recent results of Nikkilä et al (1978b) red with champion class long distance runners, yr printers. Because of the marked inter-indi- l variation, statistically only a tendency to- a higher values was found in the present study the result have to be confirmed by studying effect of controlled training program in pre- viously untrained subjects. These studies are in our next research program.

In postheparin plasma the relation between lipo- protein lipase activity and the level of physical training was not demonstrable However lipopro- tein lipase activity in postheparin plasma originates from several tissue pools such as adipose tissue and skeletal muscle (Robinson 1970) Accordingly a very poor correlation was obtained between post heparin plasma and adipose tissue lipoprotein lipase activities which is in agreement with earlier reports (Guy-Grand & Bigorie 1975 Taskinen & Nikkilä 1977). All these data stress the preference of using tissue biopsies rather than postheparin plasma in evaluating lipoprotein lipase activity in vivo.

The differences in adipose tissue lipoprotein lipase activities between the groups cannot be explained by different relative weights of the sub- jects, because this enzyme activity is known to be independent on the degree of obesity when calcu- lated on weight unit basis (Pykälistö et al 1975 Taskinen & Nikkilä 1977 Lithell et al 1978).

It is of interest in this connection that a positive correlation between adipose tissue lipoprotein lipase activity and plasma HDL-cholesterol con- centration has been reported in recent studies of Nikkilä's group (Nikkilä et al 1978a and 1978b Taskinen & Nikkilä 1979). In addition persons with high physical activity at work (Lehtonen & Viikari 1978) or in leisure time (e.g. Carlson & Mossfeldt 1964 Wood et al 1976) are known to have elevated HDL-cholesterol levels in the circulation, and high

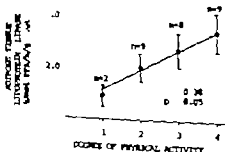


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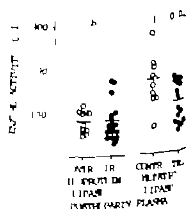


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HDL-cholesterol concentrations are generally considered protecting against coronary heart disease (cf Nikkilä 1953 Gordon et al 1977) Thus by increasing the lipoprotein lipase activity by physical training there might be a possibility in this way to accelerate the favourable removal of triglycerides from the circulation connected to the increment of HDL-cholesterol

However the exact mechanism between HDL cholesterol and lipoprotein lipase if any is still unknown and more detailed studies have to be done to ascertain it The importance of lipoprotein lipase as a regulator of triglyceride metabolism is further emphasized by the finding that lipoprotein lipase activity is the only metabolic abnormality associated with obesity that does not become normal after weight reduction (Schwartz & Brunzell 1978)

The physiological role of hepatic lipase is not yet fully understood According to a recent study by Kuusi et al (1979) hepatic lipase might be involved in the uptake of LDL and HDL-cholesterol into the liver causing perhaps an increase in the densities of these particles Thus a shift from HDL_2-subfraction to more dense HDL_3-subfraction could occur due to the action of this lipase It has been suggested that HDL_2-subfraction is a better predictor of the development of coronary heart disease (Miller & Miller 1978) The decreased hepatic lipase activity in the training group obtained in this study could thus have a beneficial effect on the distribution of the two HDL subfractions However the clarification of the possible relationship between hepatic lipase and physical activity awaits for the future

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LBRO and B LISANDER

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DELBRO D & LISANDER, B Non-ganglionic cholinergic excitatory pathways in the sympathetic supply to the feline stomach—an efferent system or afferent with excitatory axon collaterals Acta Physiol Scand 1980, 110 137 144 Received 28 Dec. 1979 ISSN 0001-6772. Department of Physiology University of Göteborg, Sweden.

Experiments were performed on chloralosed, adrenalectomized cats, paralysed with gallamine and artificially ventilated. Gastric motility was recorded by the balloon method. Efferent stimulation of the cut greater splanchnic nerv well proximal to the celiac ganglion, could either increase or decrease gastric tone The xcitatory responses called for higher stimulation intensities than the inhibitory ones but were as rule observed at lower frequencies only (1–4 Hz) They could be abolished by atropine but were not prevented by bilateral vagotomy hexamethonium nor guanethidine. The latter two drugs rather reversed inhibitory responses to excitatory ones which exhibited hyperbolic frequency-response relationship with maximal effect already at 2–4 Hz. Heating of nerve trunk selectively activates th afferents of the delta group and C-class. Heating of the greater splanchnic nerve caused an increase in gastric motility which like that caused by electric stimulation was not prevented by hexamethonium nor guanethidine nor was it eliminated by cutting the nerve centrally nor by vagotomy hile it was abolished by atropine These results suggest that the excitatory gastric responses to efferent splanchnic nerve stimulation are due to antidromic activation of thin afferent fibres. Their functional significance remains obscure but their peripheral arborizations may convey xon reflexes influencing gastrointestinal motility

Key ords Gastric excitation, sympathetic, atropine sensitive axon reflexes

rent splanchnic nerve stimulation can decrease ic tone by an activation of the adrenergic fibres ness & Costa 1974). However atropine sensi- excitatory responses have also been re ed (cf Thomas & Baldwin 1968) and in the ent series of xpts. these less studied tatory effects were further analysed A prelimi- report has been given (Delbro & Lisander 9)

THODS

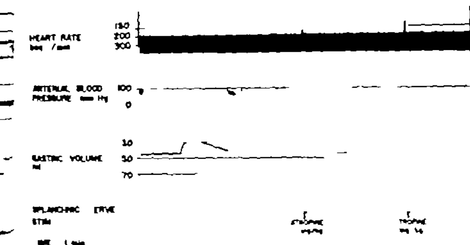
ats eighing 1 95–6 5 kg were used The animals deprived of food 24–36 h before the expts. After ction th ether they were anesthetized with oralose 50 mg/kg b wt i A tracheal cannula was rted An i infusion (0 10–0 15 ml/min) of 10–20 mEq HCO_3/100 ml, dissolved in 10 % glucose solution, was en to counteract acidosis (Haglund 1973). After midline laparotomy the adrenals were ligated to eliminate the re lease of catecholamines to the blood stream. Temperature was kept at 37 38°C by mean of heating pad.

Recording of effector response

Gastric olume was in most expts. recorded with a large rubber balloon introduced via the esophagus (cf Jansson 1969). Intragastric pressure was in the individual expts kept constant at 5–17 cm H_2O The system was filled with body warm water and the balloon was connected to container placed on Statham strain gauge recording the weight of the water outside the tomach In this way changes of gastric volume could be continuously followed at largely constant transmural pressure.

In few expts. however the volume of the tomach wa recorded by pyloric cannula (Jansson & Martinson 1966).

Blood pressure was recorded by Statham P23 AC transducer connected to catheter in femoral artery *Heart rate* was in some expts. recorded on Grass tachograph unit, triggered by the arterial pulse wave All recordings were made on Grass polygraph.



Continuation from Fig. 1. The excitatory gastric response to splanchnic stimulation is considerably reduced by ... 0.1 mg/kg, and is completely abolished by 1 mg/kg

...sed by submaximal efferent vagal stimulation ..., 5–10 V), excitatory responses could still be ...ed by splanchnic nerve stimulation (4 expts.) ...was also the case if gastric relaxation first ...nduced by activation of the ...agal high-...hold non-adrenergic non-cholinergic fibres (2 ...0 V, 1 expt., cf. Jansson 1969).

responses were observed and then at higher stimulation frequencies as well (see below).

One cat was pretreated with reserpine 3.8 mg/kg s.c. 24 h prior to the expt. In this animal excitatory effects only were observed upon splanchnic

...ts of drug

...ost expts. the muscle relaxant, gallamine 4 ...g, was repeatedly given. However also prior ...s drug excitatory (6 expts.) as well as inhibi-...(7 expts.) gastric responses could be elicited by ...ent splanchnic stimulation.

...anethidine, 3.3–20 mg/kg abolished the in-...ory responses to splanchnic nerve stimulation ...pts.) but did not counteract the excitatory ones ...h was clear from the fact that this drug did not ... the responses in animals where the inhibitory ...onses had been abolished by hexamethonium (7 ...s., see Fig. 1).

...examethonium, 5–100 mg/kg abolished or ...tly diminished the inhibitory responses (7 ...ts.). The drug could in this way unmask excita-... responses. If given after guanethidine it did ...diminish the excitatory effects (4 expts.). In one ...eriment, the ganglionic blocker trimethaphan 1 ...kg also abolished the inhibitory splanchnic re-...nses and revealed excitatory ones. After ...amethonium and guanethidine given in com-...ation (18 expts.) or separately only excitatory

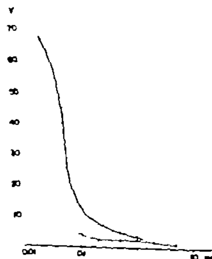


Fig. 3. Cat 2.45 kg. Threshold intensities to elicit inhibitory (dashed line) and excitatory (solid line) gastric responses by efferent left splanchnic stimulation (10 Hz). The excitatory responses were obtained after guanethidine and hexamethonium (both at 5 mg/kg). At each pulse duration one subthreshold and one suprathreshold voltage was determined, as indicated in the figure.

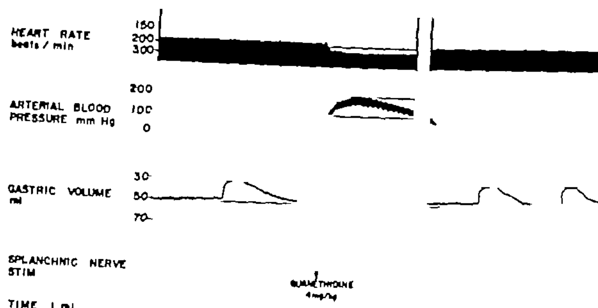


Fig 1 Cat 3.5 kg treated with hexamethonium 5 mg/kg Efferent left splanchnic nerve stimulation (4 Hz, ... before and after guanethidine 4 mg/kg The time between the panels is about 70 min

Nerve stimulations

The vagi were usually dissected free in the neck and put on strings so that they could be cut in the course of the expts Efferent vagal stimulation was accomplished by ring electrodes slipped over the cut nerve ends The greater splanchnic nerve mostly on the left side was carefully dissected free usually in the abdomen centrally cut and the distal end arranged for efferent stimulation with a ring electrode Care was taken to keep the electrode site at least 10 mm proximal to the celiac ganglion Nerve stimulations were performed with a Grass S5 stimulator or a specially constructed stimulator both delivering unidirectional rectangular pulses

Graded heating of the greater splanchnic nerve was performed by circulating warm water through a metal block with a groove containing the nerve Metal blocks of different widths viz. 3 6 and 8 0 mm, were used The warm water was heated in a thermostated bath and delivered by a circulation pump The temperature of the metal block was recorded on the Grass polygraph by a thermistor One of the heating units also contained electrodes for electrical stimulation of the same nerve section The splanchnic nerve to be heated was cautiously dissected free but was either left intact or cut proximally When the greater splanchnic nerve was to be dissected in the chest the thorax was opened laterally by resection of two or more ribs

Drugs

In most expts. artificial respiration was maintained with a respiration pump and gallamine triethiodide (Flaxedil May & Baker) was repeatedly given to prevent somatomotor interference Other drugs used were atropine (atropine sulphate Merck) hexamethonium (hexamethonium chloride Fluka) eserine (physostigmine salicylate Merck) trimethaphan (Arfonad Roche), all dissolved in isotonic saline further guanethidine (Ismelin CIBA) hydrocortisone (Solu-Cortef Upjohn ... (Nalone Endo) and reserpine (Serpasil, CIBA) ... are expressed in terms of the salts and were ... except for reserpine which was injected s.c. ... the expt and hydrocortisone which was given ...

RESULTS

Inhibitory effects of splanchnic nerve stimulation

Efferent electrical stimulation of the ... splanchnic nerve regularly decreased gastric ... In the vagally decentralized stomach the latency ... onset was more than 10 s This was also the c... splanchnic stimulation was performed ag... background of vagally induced increase in ... tone

Excitatory effects of splanchnic nerve stimulation

Efferent splanchnic nerve stimulation could increase gastric tone provided that the st... strength was high enough (see below) ... treatment with autonomic blocking drugs, ex... tory responses were most pronounced at sti... tion rates below 8 Hz Their latency of onset ... about the same as that of the inhibitory resp... and not seldom the changes in gastric tone ... biphasic with excitation preceding inhibition ... excitatory responses were not prevented by ... otomy in the neck (8 expts) If gastric tone ...

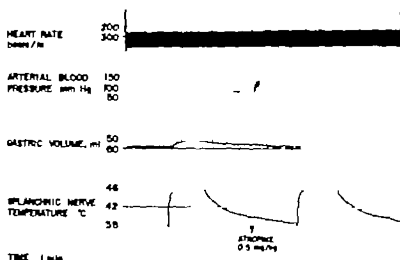


Fig. 5. Cat 3.25 kg, treated with guanethidine 3.4 mg/kg and hexamethonium, 20 mg/kg and bilaterally vagotomized. Heating of the intact left splanchnic nerve in the abdomen causes gastric excitatory response which is prevented by atropine 0.5 mg/kg.

or the frequencies that led to a maximal re (2–4 Hz). At still higher stimulation frequen- he rate of increase in gastric tone was pro- vely more rapid. It was noted throughout the that the excitatory responses to splanchnic stimulation could be kept quite stable during lations at 4 Hz or below for up to min. At 8 above there was, however an 'escape' in the me, more pronounced the higher the frequen- Furthermore, it was often observed that stimu- at 8 Hz or above could decrease the mag- of the response to a subsequent stimulation. ect, repeated stimulations at high frequencies considerably diminish the subsequent ex- ory responses.

ts of heating of the planchn nerv

ing of a nerve selectively activates afferent fibres of the A delta group and C class 1947). It was considered of interest to y the effects of heating of the splanchnic nerve ucidate whether the excitatory splanchnic ef might be due to an antidromic activation of ent fibres. Heating of the splanchnic nerve in abdomen or in the thorax, regularly and repro- bly (7 expts.) elicited an increase in gastric Temperatures up to 46°C were required. The onses occurred with a fairly short latency of on- (see Fig. 4). Like the excitatory responses to electric splanchnic nerve stimulation they were nei- the prevented by vagotomy in the neck (5 expts.), nor by guanethidine 3.3–10 mg/kg (3 expt), nor hexamethonium 5–10 mg/kg (4 expts), nor by these drugs in combination (5 expts.). Atropine however abolished these responses at the same dose level which blocked the electrically induced excitatory responses 0.1 1.0 mg/kg (5 expts.) (Fig. 5).

In 4 e pts the splanchnic nerve was left with intact central connections. Then heating of the nerve elicited signs of reflex activation of sympathetic efferents. For example pressor responses regularly occurred which could be essentially blocked by guanethidine 3.4–4 mg/kg and hexamethonium, 5 mg/kg (? expts.). Administration of these drugs enhanced however excitatory gastric responses to heating of the intact nerve (3 expts.). In no case were gastric inhibitory responses induced by heating of the nerve when reflex effects had been prevented by cutting th nerve centrally indicating that heating never activated efferent inhibitory pathways directly. On the other hand, prior to the administration of autonomic blocking drugs and vagotomy heating of the intact nerve did not elicit gastric contraction. Instead there was often a tendency towards gastric relaxation. Cutting the vagi abolished the slight relaxatory responses to heating and reversed them to contractions (xpts.)

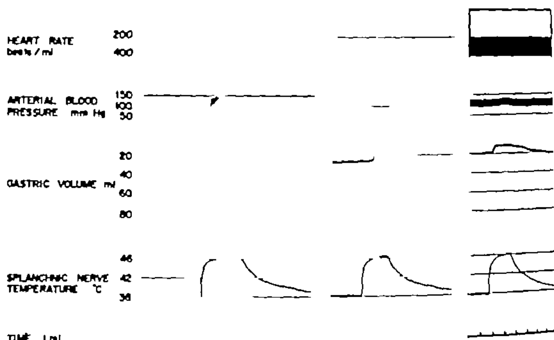


Fig 4 Cat 8 kg Heating of the intact left splanchnic nerve in the abdomen First panel The heating causes a pressor response as long as the vagi are intact Second panel After bilateral vagotomy in the neck heating causes gastric excitation Third panel After hexamethonium 5 mg/kg splanchnic heating still causes gastric excitation but previously observed reflex pressor responses are virtually abolished

nerve stimulation (2–32 Hz 1 ms 10 V) Atropine at a dose of 0 1–1 mg/kg completely abolished the excitatory effects in all instances (6 expts see Fig 2) When this drug was given following guanethidine and hexamethonium no reversal to inhibitory effects to splanchnic stimulation was observed indicating that the adrenergic effects on gastric motility had previously been fully blocked In two expts naloxone 0 015–0 080 mg/kg was given but had no effect on the excitatory responses following treatment with guanethidine and hexamethonium Eserine was given in 2 expts 0 2 and 0 5 mg/kg An augmentation of the gastric excitatory responses as well as a decrease in the threshold for their elicitation were then noted In 3 cats treated with hydrocortisone 5 mg/kg i m to compensate for the elimination of adrenocortical secretion the excitatory gastric responses to splanchnic stimulation were of essentially the same character as in the other expts

The effects of variation of stimulus intensity

In 4 expts the left splanchnic nerve was stimulated at various intensities before and after administration of both guanethidine and hexamethonium 5 and 10 mg/kg respectively The aim was to establish the threshold intensities needed to elicit inhibitory and excitatory gastric responses Strength duration curves were drawn on the basis of the observations and Fig 3 shows the result of one representative expt The thresholds for the inhibitory responses were about the same regardless of whether or not the cut vagi were simultaneously stimulated in the neck in order to accentuate the prevailing gastric tone (1 expt) The observations clearly indicated that decidedly higher stimulus rates were required to elicit the sympathetic excitatory effects than the inhibitory ones

Frequency response relationships

In 3 cats given both guanethidine and hexamethonium 5–20 mg/kg respectively the magnitude of the excitatory responses was determined at various frequencies of stimulation (0.5 1 … and 16 Hz) In additional expts the greater splanchnic nerve was stimulated with higher frequencies 10–3… Hz Responses could be obtained already at 0 5 Hz in one expt but in the others excitatory effects required at least 1 Hz When the frequency was increased maximal responses were obtained already at 2–4 Hz and still higher rates … tended to decrease the magnitude of the response At low frequencies giving submaximal responses there was a slow build-up of tone before a … contraction level was reached The same …

by conventional ganglionic blocking Several explanations to this may be offered. ossible ganglionic synapses in the pathways tudy may have been located proximal to the ting electrode Alternatively the phenome have been due to activation of exceptionally ibre preganglionic efferents with a transmit chanism resistant to conventional ganglionic s However a third possibility is that the ory responses were due to antidromic activa- afferent fibres. To elucidate the latter possi- experiments with heating of the splanchnic were performed. Heating of a nerve activates ts of the A delta group and C class (Euler Heating of the planchnic nerve to 46°C in domen or in the thorax, elicited gastric con-

responses showed the same pharmacological ties as the electrically induced excitations y were not blocked by guanethidine nor ethonium but by atropine The blocking ef atropine suggests that these afferent ramif may release acetylcholine alternatively that ctivate intramural cholinergic neurons via a itter substance resistant to conventional onic blockers

ting of the centrally cut nerve did not activate ory gastric fibres However with the nerve there were regularly indications that heating d afferent nerve fibres, producing widespread activation of visceral efferents For example relaxation was sometimes observed if the nerves were still intact. Vagotomy reversed relaxatory responses to contractions. After lanchnic nerve stimulation causes a reflex tion of the vagal non-adrenergic non- ergic relaxatory fibres (Abrahamsson, Glise se 1979). The present observations suggest ra effect involves activation of thin splanch- ferents. Furthermore heating of the intact chnic nerve regularly induced pressor re- es which could be essentially abolished by thidine and hexamethonium These pressor nses were evidently indications of widespread sympathetic excitation.

n the present experimental evidence sts that the excitatory gastric responses to nt sympathetic stimulation are due to anti- c activation of thin afferent nerve fibres. The hat these effects are effectively blocked by ine may possibly indicate that acetylcholine can be released from afferent neurons. Another alternative is that the afferents act via a nonnicotinic nonadrenergic transmitter on intramural chol nergic neurons to elicit the contraction response

The functional significance of these excitatory gastric effects by afferents is obscure One possibility is that they are involved in xonal reflexes within the stomach If so since thin visceral afferents can be activated by noxious stimuli, we suggest that they may play a role for the local pathophysiological manifestations in conditions like gastritis or gastric ulcer

The present study has been supported by grants from the Swedish Medical Research Council (14X-04249), Bergvall Foundation Groschinsky's Foundation, Jean vons Foundation, the S edish Society for Medical Research and the Medical Faculty of Göteborg Thank are due to Mrs Ann Wik tröm for xcellent technical assistance

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In ? expts heating and electrical stimulation were performed on the same part of the centrally cut splanchnic nerve Although the responses were qualitatively similar heating did not induce excitatory effects of the same magnitude as those elicited by supramaximal electrical stimulation

DISCUSSION

Both inhibitory and excitatory gastric responses could be elicited by efferent splanchnic nerve stimulation In the vagally decentralized stomach inhibitions were slow in onset and not appreciable except at higher rates of stimulation This is compatible with the concept that the inhibitory adrenergic sympathetic fibres to the gastrointestinal tract exert their main action not on the smooth muscle cells but on intramural excitatory neurons in series with the vagal motor pathways (cf Furness & Costa 1974) However the latencies to splanchnic nerve stimulation were considerable even during efferent vagal stimulation in contrast to the brisk inhibitions occurring during activation of the spinal intestino gastric reflex (Jansson & Martinson 1966) or hypothalamic stimulation (Jansson Lisander & Martinson 1969) These observations may per se suggest that the direct nerve stimulation activates another fibre population than that involved in reflexly or centrally induced sympathetic inhibitory responses The interest was here focused on the less studied excitatory responses to splanchnic stimulation If no autonomic blocking drugs had been given these responses were usually most pronounced at stimulation rates below 8 Hz and at higher frequencies biphasic or purely inhibitory responses were observed The cholinergic mediation of these excitatory responses was evidenced by the blocking effect of atropine in consonance with findings by others (cf Thomas & Baldwin 1968) The cholinesterase blocking agent physostigmine increased the responses and lowered their threshold of elicitation

Further the excitatory responses were not counteracted by the presynaptically active adrenergic blocking agents guanethidine and reserpine Rather by abolishing the inhibitory gastric responses these drugs unmasked or further augmented the excitatory responses (see below)

The splanchnic nerve stimulations were always performed well proximal to the celiac ganglion In spite of this the ganglionic blocking agents hexamethonium and trimethaphan could [illegible] the excitatory responses The inhibitory [illegible] however were completely abolished by these at low dose levels. If hexamethonium [illegible] after adrenergic blockade with guaneth[illegible] excitatory responses were not diminished

Thus the pharmacological analysis [illegible] that the inhibitory responses to direct [illegible] nerve stimulation were due to activa[illegible] ganglionic fibres, exciting postganglion[illegible] renergic neurons. The excitatory responses [illegible] other hand apparently did not involve [illegible] with conventional ganglionic transmission [illegible] be blocked by the muscarinic blocker [illegible]

Stimulation of the splanchnic nerves at [illegible] intensities established that the inhibitory [illegible] citatory gastric responses were mediated b[illegible] rate fibre populations with clearly different [illegible] tion thresholds Thus lower intensities [illegible] cient to elicit inhibitory effects which [illegible] with an activation of preganglionic [illegible] fibres The excitatory effects required [illegible] higher stimulation intensities and the thresh[illegible] ues agree well with those reported for [illegible] (Koslow Bak & Li 1973 Öberg & Thorén [illegible] should be borne in mind however that [illegible] responses rather than action potentials [illegible] used as the indicator of nerve fibre activation [illegible] present expts. Several nerve fibres may need [illegible] activated to induce clear effector responses [illegible] present approach can therefore be expected [illegible] higher threshold values than when single [illegible] preparations are studied Furthermore such [illegible] sible source of error might affect the two [illegible] responses to a different degree Experiments [illegible] the aid of recording the action potentials [illegible] splanchnic nerve are in progress to further [illegible] date this problem

In cats treated with guanethidine and [illegible] amethonium to block the inhibitory effects [illegible] excitatory responses could be elicited at stim[illegible] frequencies as low as 0 5 Hz. At ~4 Hz [illegible] contraction responses were obtained Such [illegible] range frequency response relationship is [illegible] for the axon reflex vasodilator arrangement [illegible] to somatic C fibre afferents (Celander & Fo[illegible] 1953) but is well below that characterizing autonomic neuro-effector systems where [illegible] frequencies usually are around 10–15 Hz (cf [illegible] kow & Neil 1971)

As mentioned the excitatory effects could [illegible]

...ulsants (benzylpenicillin and pentylenetetrazol) ...otential clamped myelinated axons from the rat

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BRISMAR, T & JEFFERYS J G R. Convulsant (benzylpenicillin and pentylenetetrazol) on potential clamped myelinated axons from the rat. Acta Physiol Scand 1980 110 145–148. Received 16 Jan 1980. ISSN 0001-6772. Department of Clinical Neurophysiology Huddinge Hospital Huddinge S eden.

The effect of two convulsants, benzylpenicillin and pentylenetetrazol, was tested in potential clamp experiments on single rat myelinated nerve fibres. 10 mM concentration did not affect the permeability properties of the nodal membrane The convulsiv action is therefore probably not mediated through changes in the xcitability of central axons.

Key words Myelinated nerve potential clamp benzylpenicillin, pentylenetetrazol experimental epilepsy

...mechanisms have been implicated in the ...ve action of penicillin (Pc) and of penty...azol (PTZ) Several lines of evidence point ... changes in ynaptic transmission Depres... inhibitory postsynaptic potentials has been ...ed, for instance in Aplysia neurones (2 mM ...TZ Pellmar & Wilson 1976, 1977), and also ...ocampal pyramidal cells (c.3 4 mM Pc ...ine & Gjerstad 1980). Pc increases the ex...y of presynaptic terminals or myelinated ... several excitatory projections in mammals ...ally in various thalamo-cortical paths (Gut... Prince 1972, Rosen et al 1973 Scobey & ...1975 Schwartzkroin et al 1975 Noebel & ...1978a b), in the pyramidal tract (Louvel & ...1978), and in the nerve muscle preparation ...(2 5–8.5 mM Pc, Noebels & Prince 1977) ... studies on hippocampal slices, have ...ted that the properties of the non-synaptic ...ic membrane have been modified by Pc ...y involving regenerative calcium currents or ...(Prince & Schwartzkroin 1978 Andersen ...ad & Langmoen 1978) and so causing the ...rane potential shift in positive direction which ...ble those initially described in vivo by Mat... & Ajmone-Marsan (1964)

...excitability of the postsynaptic spike gen...l mechanism has been increased by rather ...oncentrations of each drug PTZ in the range of 25–170 mM elicited spontaneous firing in molluscan neurones, which was related to a reduction in the threshold of the initial regenerati e inward current of the action potential and to a decrease in the subsequent steady state outward current (Klee Faber & Hers 1973 David, Wilson & Escueta 1974 Williamson & Crill 1976). In the frog node of Ran ier sim lar changes caused by low external calcium and added tetraethylammonium respectively also resulted in repetitive firing (Bergman Nonner & Stämpfli 1968) Application of Pc (34–84 mM) to slices of olfactory cortex was reported to increase postsynaptic excitability specifically (Voskuyl & ter Keurs 1978).

The present study examines the effect of convulsive doses of Pc or PTZ (10 mM each) on the ion currents at the nodes of Ranvier of fibres isolated from rat sciatic nerve

METHODS

Single myelinated nerve fibres (10–12 μm in diameter) were isolated from the sciatic nerve of albino rats (Sprague Dawley 250–300 g female) A single fibre was mounted in a recording chamber and connected to the electric feed back system for recording or clamping mem-

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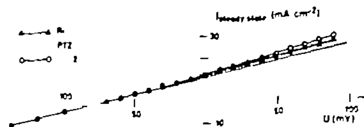


Fig. 3. Steady state membrane current vs. potential relation in Ringer 10 mM PTZ Ringer and after wash out. Same experiment as in Fig. 2.

permeability increase and the relatively large conductance (Brismar 1980) were unaffected.

USSION

investigation has shown that Pc and PTZ, in concentrations considerably greater than ed to induce epileptiform discharges in the have no effect on the potential-dependent abilities of myelinated fibres from the sciatic of rat. If as seems likely excitation in central nated axons has similar properties, then it bly does not mediate the convulsive actions of d PTZ. It is also possible that the action poten- mechanism at the trigger zones of central nes resembles that at the nodes. If that were ase the convulsive effects of the two drugs d probably not be due to increased excitability se postsynaptic sites.

e possible action of two widely used convul- benzylpenicillin (Pc) and pentylenetetrazol), on the nodal membrane was tested in poten- lamp experiments on single myelinated nerve from the sciatic nerve of the rat. The specific permeability properties associated with the anism for impulse conduction were analysed. M Pc or PTZ in Ringer's solution had no sig- ant effects on the Na permeability mechanism the permeability at steady state. The present ngs make it unlikely that the convulsive action and PTZ is related to changes in the electrical erties of excitable membranes as measured at node of Ranvier.

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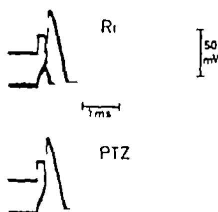


Fig 1 Membrane action potentials (and subthreshold responses) in rat myelinated nerve fibre in Ringer s solution and test solution with 10 mM PTZ Ringer Temp 28°C

brane potential Thi method and the principles for calibration and analysis of the membrane currents ha e been described elsewhere (Brismar 1980) The potential clamp technique is similar to that developed by Dodge and Frankenhauser (1958) for the analysis of frog myelinated nerve fibres

Test solutions with benzylpenicillin (Kabi) and pentylenetetrazol (Sigma) were prepared by adding these substances to the ordinary Ringer s solution (147 mM NaCl 5 9 mM KCl 3 0 mM $CaCl_2$ 5 0 mM Trizma® buffer of pH 7 4 at 25°C)

RESULTS

Membrane action potentials were re[illegible] Ringer s solution and after 5 min in [illegible] Ringer and no change was detected threshold the amplitude and the shape of the potential There was neither any effect on [illegible]ing potential which remained at the level to which it previously had been set by the balance control

The membrane currents were recorded [illegible]tential clamp conditions. Step changes in potential were made from resting level ([illegible] various values (U) and the consequent [illegible]rent was plotted against the amplitude of the Fig 2) The membrane currents and the [illegible]bility properties of normal rat myelinated fibres are described in Brismar (1980) [illegible] (Fig 2) and similarly 10 mM P[illegible] had no [illegible] the curve of initial current vs. potential [illegible] time course (τ_h) and steady state level of sodium inactivation

The steady state currents associated [illegible] changes in potential are plotted in Fig 3 [illegible] PTZ or 10 mM P[illegible] in Ringer s solution [illegible] the amplitude of the steady state current [illegible]ing that the normal low possibly K [illegible]

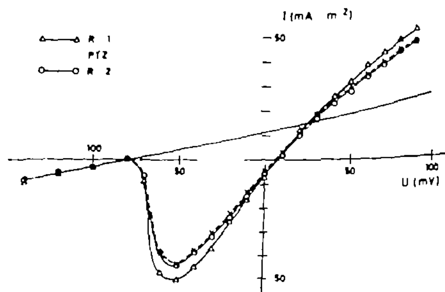


Fig 2 Peak initial current vs potential curves in Ringer's solution after 5 min in 10 mM PTZ Ringer and after wash out Current amplitude measured after step change in potential to different values as indicated on abscissa. Holding potential −80 mV P_{Na} inactivation removed by ms conditioning pulse to −120 mV Smooth curves fitted to symbols Leak current extrapolated from measured inward current at negati e steps. Temp 26°C.

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...l cerebral glucose consumption during insulin induced ...oglycemia and in the recovery period following ...ose administration

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ABDUL RAHMAN A. & SIESJÖ, B K. Local cerebral glucose consumption during insulin-induced hypoglycemia, and in the recovery period following glucose administration. Acta Physiol Scand 1980, 110 149-159 Received 17 Jan 1980, ISSN 0001-6772. Laboratory of Experimental Brain Research and Department of Neurosurgery University of Lund, Sweden

Using the ^{14}C-deoxyglucose technique we estimated local glucose consumption in the rat brain (l-CMR_{gl}) in hypoglycemia of sufficient severity to cause cessation of spontaneous EEG activity and in the recovery period following 30 min period of such hypoglycemia. After 5 and 30 min of hypoglycemia, l-CMR_{gl} was markedly reduced in many cerebral structures (cerebral cortices, caudateputamen, thalamus, and hippocampus) but unchanged or only moderately reduced in other structures (cerebellar cortex hypothalamus, and pontine grey). The results indicate that differences in l-CMR_{gl} were caused by regional differences in the true or apparent kinetic constant for glucose transport, or that the consequences of hypoglycemic derangement of cellular metabolism interfered with glucose transport in some regions. Local CMR_{gl} was markedly heterogenous in the recovery period induced by glucose administration in animals with a prior (30 min) period of hypoglycemia. In general, reduced posthypoglycemic glucose consumption was correlated to low l CMR_{gl} values during hypoglycemia. However the hippocampus provided an exception since its CMR_{gl} returned to control values. A correlation with previous measurement of local cerebral blood flow (l-CBF) demonstrates that regions lacking pronounced reduction in l-CMR_{gl} during hypoglycemia developed mismatch between blood flow and metabolic rate in the recovery period the flow showing disproportional reduction

Key ords: Hypoglycemia, brain metabolism local glucose consumption

...urements of cerebral blood flow (CBF) and of ...ovenous differences in glucose and oxygen ...nt during hypoglycemia have demonstrated ...cerebral glucose consumption (CMR_{gl}) is re...d to a much larger extent than oxygen con...tion (CMR_{O_2}) indicating that endogenous sub...es are used to support energy production (Kety 1948, Eisenberg & Seltzer 1962 Della Porta et ...964 Gottstein & Held 1967 Pappenheimer & ...bell 1973 Norberg & Siesjö 1976) When the ...glycemia is severe enough to produce cessa... of spontaneous electroencephalographic (EEG) ...als and clinical coma CMR_{O_2} is, at least in...y upheld at control levels (Della Porta et al ...l Norberg & Siesjö 1976) However energy ...duction is nevertheless insufficient to cover the ...ds, and one observes marked perturbation of ...rgy state with decreases in the tissue concen...tions of phosphocreatine (PCr) and ATP and increases in those of ADP and AMP (Hinzen & Müller 1971 Lewis et al. 1974*a* Norberg & Siesjö 1976)

Part of the endogenous substrate that is mobilized in hypoglycemia emanates from the tissue stores of glycolytic metabolites citric acid cycle intermediates and free amino acids (Goldberg et al 1966 Lewis et al. 1974*b* Norberg & Siesjö 1976 Agardh et al 1978). However evidence exists that severe hypoglycemia leads to consumption of structural components as well Decreases in tissue concentrations of lipids and proteins were observed when isolated cat brains were perfused with glucose free blood (Abood & Geiger 1955) In intact animals, decreases in phospholipid components have been noted (Hinzen et al. 1970, Agardh et al in preparation) Presumably degradation of intracellular membranes contributes to the irreversible neuronal damage that occurs in severe pro-

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..l cerebral glucose consumption during insulin-induced ..glycemia and in the recovery period following ..ose administration

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longed hypoglycemia (Meyer 1963 Brierley et al 1971 Agardh et al 1980)

Previous information on cerebral blood flow and metabolic rates in hypoglycemia pertains to the whole brain or to the cerebral cortex Since neuronal damage after hypoglycemia shows preferential localization to certain regions (mainly cerebral cortex and hippocampus) it seems highly warranted to obtain information on regional blood flow and glucose consumption Recently we described changes in local CBF during hypoglycemia and in the recovery period following glucose administration (Siesjö & Abdul-Rahman 1979 Abdul Rahman et al 1980) The results showed that during severe hypoglycemia CBF increased 2 to 3-fold in all structures visualized by the autoradiographic technique and that a pronounced hypoperfusion developed in many regions in the posthypoglycemic recovery period The present study concerns local glucose consumption (l-CMR_{gl}) as measured with the autoradiographic ^{14}C-deoxyglucose technique of Sokoloff et al (1977) The study was designed to provide answers to the following questions (1) Is there a uniform decrease in local CMR_{gl} in the brain during moderate and severe hypoglycemia or are regional differences observed? This problem was approached by measurements of l CMR_{gl} in insulin treated rats that had either no EEG changes an EEG pattern of slow waves polyspikes or cessation of spontaneous EEG activity for 5 or 30 min (2) Is local CMR_{gl} during hypoglycemia determined by the glucose availability i e by blood flow and arterial glucose concentration or are additional mechanisms involved? In answering this question data for l-CMR_{gl} were correlated to previous results on l-CBF (3) Is there a posthypoglycemic depression of l-CMR_{gl} corresponding to the reduction in local CBF? If this is so is the depression of metabolic rate uniform or is it confined to certain regions? In the experiments performed to answer this question animals were given glucose after a 30 min period of hypoglycemic "coma" and l-CMR_{gl} was measured 90 min later

METHODS

1 *Operative and sampling techniques*

The experiments were performed on male Wistar rats, weighing 315–410 g The animals were starved for 24 h but had free access to tap water They were then injected with insulin (Novo Actrapid Novo Industri AB) in a dose of 40 I U kg i p After 60 min the animals were [illegible]thetized with 3–3% halothane When [illegible] were tracheotomized connected to a Stark [illegible]rator paralyzed with tubocurarine chloride [illegible] i v) and ventilated on 1% halothane and [illegible] Respiration was adjusted to give arterial P_{CO_2} of 35–40 mmHg Body temperature as [illegible] rectum was kept close to 37°C by means of a heat[illegible]

Both femoral arteries were cannulated, [illegible] pressure recordings and the other for sampling for analyses of pH blood gases, glucose concentr[illegible] and ^{14}C-activity The latter catheter was cut to [illegible] 1.5–2 cm to avoid smearing One femoral vein [illegible]lated for infusion of ^{14}C-deoxyglucose and [illegible] infusion of glucose during the recovery period [illegible] plated screws were inserted through the skull [illegible] to make contact with the dura and connected [illegible] an Elema EEG machine In some animals a [illegible] hole was made over the superior sagittal sinus [illegible] allow sampling of cerebral venous blood in [illegible]laries with one end drawn out to a fine point [illegible]tive procedures were completed halothane [illegible] continued and the animals were ventilated on [illegible] and 30% O

Experimental group

Following the injection of insulin in a dose [illegible] I U kg blood glucose concentrations are [illegible] reduced In the artificially ventilated rat, the [illegible] hypoglycemia leads to typical EEG changes, [illegible] an increased incidence of slow waves, [illegible] slow wave polyspike pattern and ending [illegible] spontaneous electrical activity (Lewis et al 1974 [illegible] present study the animals were grouped according [illegible] EEG pattern as follows

(a) *Control* The values used were those previ[illegible]tained in animals that were starved for 18-24 h [illegible] et al 1980)

(b) *Asymptomatic hypoglycemia* In [illegible] (n=4) insulin was injected after completing the [illegible] procedure and ^{14}C-deoxyglucose was infused [illegible]ma glucose concentrations had decreased to [illegible] µmol g At these concentrations the EEG [illegible] normal and remained so during the time of sampl[illegible]

(c) *Moderate hypoglycemia* In these animals [illegible] EEG showed a markedly increased incidence of [illegible] waves at the time of the ^{14}C-deoxyglucose-infusion [illegible]ma glucose concentrations were in the range [illegible] µmol g

(d) *Severe hypoglycemia* All animals in this group [illegible] cessation of EEG activity for either 5 min (n [illegible]) or [illegible] (n=4) before infusion was begun. Plasma glucose con[illegible]centrations ranged between 0.58 and 1.26 µmol [illegible]

Four separate animals were allowed a 20 min [illegible] isoelectricity before six consecutive samples of [illegible] and cerebral venous blood were simultaneously [illegible] every second min After the last samples were [illegible] 30 min of isoelectricity") ^{14}C iodoantipyrine was [illegible] for 30 s whereafter the animal was decapitated [illegible] brain processed for measurement of local CBF

(e) *Recovery* These animals (n=4) were allowed [illegible] min period of isoelectricity" before recovery [illegible]

Physiological parameters during insulin-induced hypoglycemia and in the recovery period follow ... ose injection

: means _ S E. Plasma glucose concentration is given in ranges. MABP mean arterial blood pressure

	Temp (°C)	MABP (mmHg)	Pa_{O_2} (mmHg)	Pa_{CO_2} (mmHg)	pH	Plasma glucose (μmol ml)	EEG pattern
=6)	37 1±0 1	135±6	123 2	36.8±0 8	7.37±0.01	6.12–6.55	Normal
emia asympto- =4)	37.5+0 2	135 2	94 5	35 4 1.6	7.30+0 02	60±1.34	Normal
emia "Isoelectric (5)	37.0±0 2	115±5	108±4	34 4±1.2	7.32_0 03	0 6–1.26	Isoelectric
emia "Isoelectric 3' (=4)	36 9±0 2	108±1	114±6	33 8±1 7	7.36±0 04	0.58–1 10	Isoelectric
emia Isoelectric 5 recovery 90'	36 8±0 2	113±3	99+4	36 6 ±1	7.35 0 06	9_1 13 74	Low voltage Slow wa es

administration of glucose as an initial dose of 0.5 0% (w/v) solution, followed by constant infu- ne same solution at rate of 1 ml h for 90 min et al 1978). At that time ^{14}C-deoxyglucose was

...urement of local CMR and local CBF

CMR$_{gl}$ was measured with the quantitative au- graphic technique of Sokoloff et al. (1977). Details cedures have been given in previous com- tion from the laboratory (Ingvar et al. 1980). In y solution containing ^{14}C-deoxyglucose (337 mol^{-1} New England Nuclear) was infused i der The total dose injected as 25 μCi in hypo- ic animals and 50 μCi in recovery animals. During min period of measurement, 14 timed 100 μl arterial were taken (including blank). The samples were ately centrifuged and the plasma was separated zen at 80°C for later determination of ^{14}C-activity cose concentration. After the last (30 min) sample mal was decapitated, thereafter the brain was re- and frozen in Isopentane chilled to −50°C with nitrogen. Plasma glucose concentration were ed with an enzymatic, fluorometric method based hexokinase reaction, and ^{14}C activity with liquid ation techniques. The ^{14}C activity in the brain was ed by applying 20 μ sections to X-ray films with uent densitometric measurements. By the use of mate standards the tissue ^{14}C-activity could be cal- l.

n calculation of l-CMR$_{gl}$ according to Sokoloff et 77) "lumped constant" is used which takes into t, among other things, the K$_m$ and V$_{max}$ values for ort of glucose and ^{14}C-deoxyglucose between blood sue. Under normal conditions the value of this nt is 0 48 (Sokoloff et al 1977). In hypoglycemia, nstant shows an inverse relationship its plasma e concentration (Sokoloff et al., work in progress) koloff was kind enough to provide us with pro- al values for plasma glucose concentrations of about l ml^{-1} (0 87) and about 3 μmol ml^{-1} (0 69). No value is yet available for plasma glucose concentrations of about 1 μmol g^{-1} i e values observed i animals with a ceased EEG activity. For these, w provisionally assumed lumped constant of 0 87 and performed separate ex periments to control that l-CMR$_{gl}$ values so obtained were reasonably lose to those expected. However as the fol

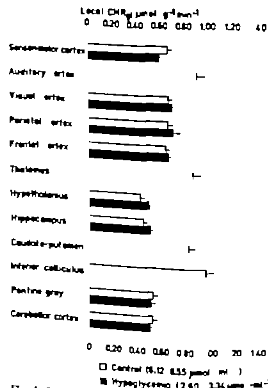


Fig. 1. Local cerebral glucose consumption (CMR$_{gl}$) in control animals and in animals with moderate insulin-induced hypoglycemia. The values are means ± S E. A significant change in CMR$_{gl}$ was observed only in the caudate-putamen ($P<0.05$).

Table ? Local glucose consumption (l-CMR g) in insulin-induced hypoglycemia

Structures	Control (n=6)	Isoelectric 5 (n=5)	Isoelectric 30' (n=4)
A *Superficial cerebral structures*			
Cortex			
1 Sensimotor	0 68±0 004	0 ?9±0 03	0 34±0 06
Auditory	0 9?±0 07	0 33±0 04	0 40±0 07*
3 Visual	0 69±0 03	0 ?6±0 03	0.29±0 05
4 Parietal	0 69±0 04	0 ?9±0 03	0.34±0 06
5 Frontal	0 67±0 03	0 ?4±0 03	0.26 0 04
B *Deep cerebral structures*			
6 Thalamus	0 89±0 07	0 43±0 04	0 35±0 06
7 Hypothalamus	0 45±0 03	0 41±0 04	0 35±0 06
8 Hippocampus	0 47±0 03	0.20±0 0?	0 ?3±0 04
9 Caudate-putamen	0 86±0 05	0 ?3±0 03	0 ?7±0 05
C *Mid brain and pons*			
10 Inferior Colliculus	1 00±0 06	0 56±0 07*	0 56±0 07*
11 Pontine gray	0.55±0 04	0 4? ±0 04	0.37±0 05
D *Cerebellum*			
1 Cerebellar cortex	0.55±0 03	0 50±0 06	0 4? ±0 09

The values are given in μmol g mm and represent means ± S E P<0 05 P<0.01 and P<0 001

lowing discussion will emphasize the l-CMR_{gl} values obtained in hypoglycemia are only approximate and the major information of the study concerns differences in l-CMR_{gl} between various brain structure (see Discussion)

Local CBF was measured autoradiographically with ¹⁴C-iodoantipyrine as the diffusible tracer (Sakurada et al 1978 for details see Abdul Rahman et al 1979)

RESULTS

Table 1 shows values for physiological parameters measured at the time of ¹⁴C-deoxyglucose infusion All groups had mean body temperatures close to 37°C arterial P_{O_2} values above 90 mmHg P_{CO_2} values in the range 31–37 mmHg and pH values in the range 7.30–7.37 In the three most severely hypoglycemic groups and in the recovery group mean arterial blood pressure was reduced However no single animal had a blood pressure lower than 100 mmHg The plasma glucose concentrations given (ranges) are the means of 14 values measured during the 30 min following C-deoxyglucose administration Also given in the table are the predominant EEG pattern observed during this period

Regional changes in CMR_{gl} during hypoglycemia

In one group of animals ¹⁴C-deoxyglucose was infused when plasma glucose concentrations were close to 3 μmol g (range 2.6–3.3 μmol g) the EEG showed an EEG pattern undistinguishable from control In all animals plasma glucose concentrations remained within narrow limits during the min period of blood sampling. Fig. 1 compares CMR_{gl} values in a control group of six animals which did not receive insulin (lumped 1980 lumped constant=0 48) to those in animals with a plasma glucose concentration of 3 μmol (lumped constant=0 69) Except for a small increase in l-CMR_{gl} in the caudoputamen could well be fortuitous the insulin-injected glycemic animals had l-CMR_{gl} values like those of the controls We conclude that the degree of hypoglycemia which is moderate enough to leave the EEG pattern unaltered does not change l-CMR_{gl}

In the moderately hypoglycemic animals with glucose concentrations 1 7?–? ?1 μmol g initial EEG pattern was one with slow waves However this functional state could not be upheld the EEG changed appreciably during the sampling period and in two animals EEG ceased In view of this unsteady state since the values obtained did not show variations in l-CMR_{gl} they will not be further considered

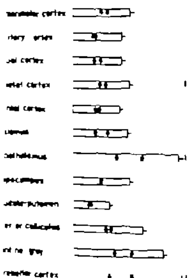
Local CMR_{gl} values obtained in isoelectric







Local [illegible] cerebral glucose consumption [illegible] hypoglycaemia (plasma glucose concentration 0.6 [illegible]) The autoradiographs [illegible] processed in the [illegible] microscanning equipment of the Laboratory [illegible] Metabolism, NIMH, Bethesda by courtesy L. Sokoloff (Goochee, Arnaud & Sokoloff 1980) provisional lumped constant of 0.87 was used the colour scale is only semiquantitative. The approximate CMR_{gl} values are for red 0.30, yellow 0.20 and green 0.15 μmol g [illegible] min. The three sections given are representative of the following levels. Top: sensorimotor cortex, caudate-putamen and lateral ventricles. Middle: Parietal cortex, thalamus, hypothalamus and hippocampus. Bottom: Cerebellar cortex.



Local cerebral glucose consumption in hypo... of sufficient severity to cause cessation of EEG ...y for either 5 or 30 min. The staples denote pooled (5 30 min) for animals ...th plasma glucose con... in the range 1 03–1 26 μmol ml⁻ (means + 7) The dots denote individual values measured ...tamals (5 and 30 min) with plasma glucose concen... of 0.58 and 0 60 μmol ml respectively I all ...eas except the hypothalamus and the cerebellar CMR_{gl} in hypoglycemia was significantly reduced ...ontrol (P 0 05)

were much more consistent and the ab... values obtained were close to those expected independent measurements (see below) In all ...ments ...xcept two plasma glucose concen... ...ons at 0 and 30 min differed by less than 20 % ...able 2 shows values obtained when ...C-deoxy... ...ose was infused 5 min or 30 min after cessation ...EG activity There were no systematic dif... ...nces between values obtained in the 5 and 30 groups Values were therefore pooled for those ...eriments in which the mean plasma glucose ...centrations fell within the narrow range of ... 1.26 μmol g (n=7). Excluded from this ...up were tw... experiments in which mean plas... glucose concentrations were 0.58 and 0 60 ...ol g Local CMR_{gl} for these two ranges of ...sma glucose concentration are given in Fig. 2 (in percent of control). The result of Table and Fig. demonstrate a marked reduction in l-CMR_{gl} in cerebral cortical structures thalamus, hippocampus and caudate-putamen a less marked reduction in inferior colliculus and pontine grey and no reduction in hypothalamus or cerebellar cortex The result also show a further pronounced reduction in l-CMR_{gl} with a fall in plasma glucose from 1 1 to 0 6 μmol ml

The results described were obtained by taking the ...erage of multiple densitometric readings from the same structure This procedure and the large aperture of the densitometer hampered resolution of localized changes in l-CMR_{gl} The results of Fig. 3 which were obtained w th the scanning densitometer of the Laboratory of Cerebral Metabolism National Institute of Mental Health Bethesda (Goochee et al 1980) offer a finer resolution. The results emphasize the low CMR_{gl} in cerebral cortical structures caudate-putamen, and hippocampus as well as the high CMR_{gl} in hypothalamus and cerebellar cortex. However they also show high glucose consumption values in structures not ...isualized by the manual densitometer (e g. septal nuclei and plexus choroideus) and a marked heterogeneity in other structures mainly the thalamus

In order to study whether calculation of l-CRM_{gl} with a lumped constant of 0.87 gives values close to the true ones regional glucose consumption wa... calculated from arteriovenous differences in glucose content and l-CBF the latter measured autoradiographically with ...C-iodoantipyrine In the calculations shown in Table 3 we assumed that venous samples drawn from the superior sagittal sinus mainly reflect blood flow in the frontal and parietal cortex and we therefore derived CMR_{gl} for these structures. In one of the experiments two AVD_{gl} values were negative and the calculated mean value was close to zero. The results of the other three experiments suggested a direct relationship between plasma glucose concentration ...nd AVD_{gl} as well as between plasma glucose concentration and CMR_{gl} (see below) In 2 expts the plasma glucose concentrations were sufficiently similar to those of the two groups of Fig. 2 to allow a comparison of CMR_{gl} values. The CMR_{gl} values within parenthesis are those measured with the deoxyglucose technique at plasma glucose concentrations of about 0 6 (n=2) and about 1 1 (=7) μmol ml⁻ As the results show there was a fu...

Table 3 *Arteriovenous differences in glucose concentration* (AVD_{gl}) *cerebral blood flow (CBF* *calculated cerebral glucose consumption* (CMR_{gl}) *related to plasma glucose concentration in hypoglycemia*

Plasma glucose concentrations and AVD_{gl} values are means ± S E (n=6) CMR_{gl} values given within parentheses are those measured autoradiographically at similar plasma glucose concentrations (see text)

Rat No	Plasma glucose (µmol ml)	AVD_{gl} (µmol ml)	CBF ml g min Frontal	Parietal	CMR_{gl} µmol g min Frontal	Parietal
1	0.34±0 07	0 04±0 01	14	69	0.09	0 11
	0 41±0 04	0 01±0 01	1.87	74	0 02	[illegible]
3	0 67±0 10	0 06±0 0	97	3 4	0 18 (0 15)	0 21 (0 17)
4	0 91±0 11	0 10±0 01	3 94	5 [illegible]	0.39 (0.30)	[illegible]

agreement between CMR_{gl} cal ulated from CBF and AVD_{gl} and those measured with the deoxy glucose technique

Local CMR_{gl} *and glucose availability*

The present results demonstrate that within any given structure CMR_{gl} was tightly correlated to plasma glucose concentration This is exemplified for frontal cortex and the hypothalamus in Fig 4 As already stated a similar relationship was obtained when CMR_{gl} was calculated from AVD_{gl} and CBF (see Table 3)

The dependence of glucose consumption on plasma glucose concentration does not explain the regional differences in CMR_{gl} Possibly such differences could reflect corresponding differences in glucose availability i e in the product of arterial glucose concentration and CBF However measurements of AVD_{gl} (see Table 3) should indicate that CBF is not an important determinant of glucose availability (and hence of CMR_{gl}) Lund Andersen 1979) Thus, since AVD_{gl} did [illegible] exceed 10% of plasma concentration the results suggest that the arterial plasma glucose concentration is the dominant factor determining glucose availability We conclude that although the plasma glucose concentration determines CMR_{gl} in a given structure differences between structures are caused by other factors (see Discussion)

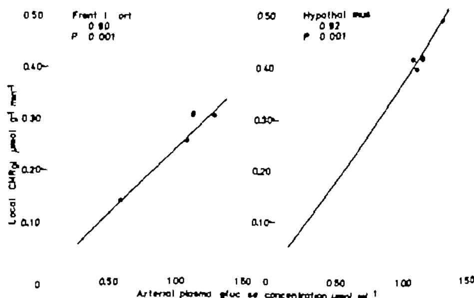
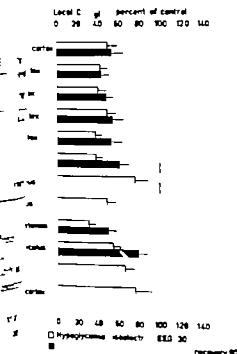


Fig 4 Individual value for glucose consumption (CMR_{gl}) in frontal cortex and hypothalamus during severe hypoglycemia related to plasma glucose concentrations The values relate to a period of hypoglycemic coma of either 5 (open circles) or 30 (filled circles) min duration



Local cerebral glucose consumption (CMR_{gl}) in ...very period following 30 min period of severe ...cemia, as related to CMR_{gl} at the end of the ...cemia. The values are means ± S.E. in percent of ... During recovery CMR_{gl} was reduced below con... (p<0.05) in all structures except the hypothalamus ...campus, pontine grey and cerebellar cortex



Fig. 6. A comparison between local cerebral glucose consumption (CMR_{gl}) and blood flow (CBF) after 90 min of recovery following 30 min period of severe hypoglycemia. The values are means ± S.E. in percent of control values. The CBF values were taken from Abdul-Rahman et al. (1980).

CMR in the recovery period

CMR_{gl} in the posthypoglycemic recovery ...as measured 90 min after the start of ...administration, following 30 min period of ...lycemia with ceased EEG activity. In Fig. 5 ...ery values for CMR_{gl} have been compared to ...measured in the 30 min hypoglycemic group. CMR_{gl} was markedly heterogenous in the ...ery period. In many structures (cerebral cor... thalamus and caudate-putamen) the values ...reduced to 45–60% of control while in others ...thalamus, hippocampus, inferior colliculus, ...ne grey and cerebellar cortex) values were ... to control. In general areas with markedly ...ed CMR_{gl} during hypoglycemia showed low ...s in the recovery period, and those having ...moderately reduced CMR_{gl} during hypo...mia showed recovery values close to con... However one notable exception was the ...ocampus whose glucose consumption rate re...d to control values in the recovery period.

The results presented raise the question whether or not the previously reported reduction in 1-CBF during the recovery period (Abdul-Rahman et al. 1980) merely reflects a reduced metabolic rate. In order to evaluate this possibility local glucose consumption rates were arranged in the order of increasing values and compared to the corresponding 1-CBF values. As fig. 6 shows, 1-CBF was reduced out of proportion to the reduction in 1-CMR_{gl} in all cortical areas and in the hippocampus there was pronounced mismatch between metabolic rate and blood flow.

DISCUSSION

The present results give novel information on local cerebral glucose consumption in severe hypoglycemia and they provide answers to the questions raised in the introduction. Before these results are discussed it seems warranted to briefly consider the validity of the deoxyglucose technique when applied to severe hypoglycemia.

The de xyglucc se technique in hypoglycemia

The unique feature of the C-deoxyglucose technique of Sokoloff et al (1977) is its ability to measure *local* glucose consumtpion In hypoglycemia glucose consumption is no longer synonymous with metabolic rate since endogenous substrates partly substitute for the exogenous glucose Thus the technique then estimates glucose delivery to structures in the brain rather than their metabolic rates In all probability glucose delivery is inversely proportional to the derangement of local cerebral metabolism caused by the hypoglycemia Information on glucose delivery during hypoglycemia and on posthypoglycemic metabolic rates seems a prerequisite for understanding the preferential localization of neuronal lesions to certain brain structures

One problem of applying the technique to hypoglycemia is that the lumped constant varies with plasma concentration (Sokoloff et al in preparation) Since the main emphasis in the present work was on regional differences in CMR_{gl} we chose to use the provisional lumped constants obtained by Sokoloff et al

In the isoelectric groups values for CMR_{gl} were reproducible presumably because plasma glucose concentrations remained within narrow limits Two findings indicate that the absolute CMR_{gl} values that were obtained with a lumped constant of 0 87 were reasonably accurate First at this degree of hypoglycemia previous calculations of CMR_{gl} from CBF and AVD_{gl} indicate a reduction of glucose consumption to about 40% of control (Norberg & Siesjö 1976) Second similar calculations performed in the present study gave CMR_{gl} values approximating those obtained with the C-deoxyglucose technique (Table 3)

Since the deoxyglucose technique gives CMR_{gl} values for a 30 min steady state period it may be asked if values obtained in the present 5 and 30 min groups really pertain to these periods of hypoglycemic "coma Two facts indicate that the values obtained are good approximations of early and late" hypoglycemic coma First CMR_{gl} values normally obtained are heavily weighted in favour of the first 15 min following deoxyglucose infusion (Sokoloff 1978) Second since severe hypoglycemia reduces tissue glucose concentrations to vanishingly small values the precursor pool must show a very rapid turnover

Regi nal differences in CMR_{gl} durin hypoglycemi

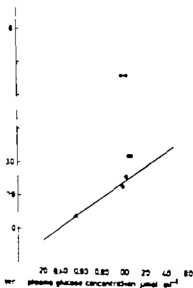
The present results clearly demonstrate ... differences in CMR_{gl} occur in severe hyp... For example in the 5 and 30 min grou... structures that normally have a low metab... (e g cerebellar cortex and pontine gr...) CMR_{gl} values greatly exceeding those ... other area (e g cerebral cortex) Two q... must be posed First does the close rel... between plasma glucose concentration and ... reflect a rate-limiting transport of gluc... blood to tissue Second if this is so, wh... local differences in CMR_{gl}? In order to ... these problems we must consider glucose ... between blood and tissue

Current information on glucose flux ... blood and brain suggests that it occurs by fa... transport via a symmetrical mobile carr... the rate-limiting step being mainly locali... blood-brain barrier (Lund-Andersen 197... Siesjö 1978) If this information is correct ... port can be described by the equation

$$\text{Net flux} = \frac{T_{max} \cdot C}{C + K} - \frac{T_{max} \cdot C_t}{C_t + K_t}$$

where C and C are the concentration of ... on either side of the blood-brain barrier T_{max} ... maximal transport rate and K is the affin... stant between glucose and transport mech... severe hypoglycemia C_t can be set to zero ... et al 1974a) Assuming a K of 8 μmol ml ... T_{max} of 2 2 μmol g⁻¹ min⁻¹ (see Borgström ... 1976) we can provisionally calculate net flux ... plasma glucose concentration range 1 ... μmol ml and compare the values obtai... those measured with the deoxyglucose met... the 5 and 30 min groups In Fig 7 data are ... for the frontal cortex and inferior collicul... results demonstrate that l-CMR_{gl} in frontal ... which exemplifies the conditions in areas ... low CMR_{gl} closely reflects influx of gluco... plasma However in colliculus inferior a re... tative high-CMR_{gl}" structure glucose co... tion greatly exceeds the expected influ... sible explanations are conveniently disc... the basis of two alternative hypothesis

(a) *The true kinetic constants show regi... ferences* Clearly the results obtained co... explained by regional variations in K and ...



Local cerebral glucose consumption (CMR_{gl}) in ortex and inferior colliculus in severe hypo- related to arterial plasma glucose concentration The line represents the expected influx of glucose transport V_{max} of 22 μmol g min and μmol ml

For example if "high CMR_{gl}" areas have T_{max} or lower K values than those pro- lly used they would tolerate a more sever of hypoglycemia than other tructures In f the fact that regional differences in glucose may determine the neuropathological out f severe hypoglycemia it seems highly war to obtain information on egional values for T_{max}

The true kinetic constant are niform hout the brain Obviously if this were the T_{max} must be higher or K lower than ed (the values must fit the areas with the CMR_{gl} values) Furthermore some factors hen have impeded glucose influx in areas with CMR_{gl} values Two possibilities could be en- d. Firstly it is conceivable that the glucose could be inhibited by products of the de metabolism e.g. free fatty acids (cf Han- 1971) Secondly if cell edema compromises ry circulation e.g. by caus ng preferential ng of blood the *effecti* T_{max} would be re

resent there is ot sufficient information a ailable to distinguish between the possibilities con- sidered and further work is requ red to explore the mechanisms underlying the pronounced regional differences in CMR_{gl}

Regional differences i CMR_{gl} in the recovery period

The present results unequivocally demonstrate that a normal CMR_{gl} in the recovery period was ob- served only in a few cerebral structures while CMR_{gl} was severely depressed in others It is tempt ng to conclude that areas with a low meta- bolic rate (e g. cerebral cortical structures) suffered functional damage during hypoglycemia pre ent ng a return of metabolic rate following glucose ad ministration. This conclusion recei es support from results on oxygen consumption which i also de pressed in the recovery period (Agardh et al In preparation) as well a from neuropathological studies which demonstrate relatively extensi e neuronal damage in the cerebral cortex (Agardh et al 1980 Kalimo et al 1980) However the present results on the hippocampus demonstrate that a marked reduction in CMR_{gl} during hypoglycemia does not necessarily prevent a return of metabolic rate to control alues

The correlation between l-CMR_{gl} and l-CBF in the recovery period provides an interesting in- sight into events of probable importance to the pathophysiology of hypoglycemic cell damage Thus areas which are considered sensiti e to hypoglycemic cell damage showed mismatch be tween CMR_{gl} and CBF the latter being reduced out of proportion to the depression of metabolic rate This was especially evident in the hippocampus whose blood flow fell to 77% of control while CMR_{gl} returned to control However since CBF was reduced to 50% of control after 30 min of recovery and to 40% after 180 min (Abdul-Rahman et al 1980) the difference i results between the hippocampus and other regions (cerebral cortex and caudate-putamen) is less pronounced than what is indicated by Fig 6

Relation to neuropathology

Carefully controlled studies of the neuropatho- logical alterations following hypoglycemia have previously only been carried out in monkeys (Brierley et al 1971) I these insulin-in- duced hypoglycemia was sustained so as to bolish somatosensory-evoked potential for 49–97 min.

Neuronal alterations occurred diffusely in the neocortex and in some animals also in the hippocampus and caudate-putamen Recently extensive studies on rats have been carried out with both light and electron microscopic techniques (Agardh et al 1980 Kalimo et al 1980) The results showed that a 30 min period of hypoglycemia with ceased EEG activity was accompanied by relatively extensive neuronal alterations in the cerebral cortex So far however the studies have not been extended to include other parts of the brain However provided that changes are similar in the monkey and the rat the present results and those of our previous study (Abdul Rahman et al 1980) indicate that nerve cell damage occurs in areas that suffer a pronounced reduction in CMR_{gl} during hypoglycemia and which show a marked mismatch between flow and metabolic rate in the recovery period

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ut of bile and pancreatic enzymes after test meals different fat content. Influence of body weight ıncreatic enzyme composition

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EKELUND, K. & JOHANSSON C Output of bile and pancreatic enzymes after test meals with different fat content. Influence of body weight on pancreatic enzyme composition. Acta Physiol Scand 1980, 110 161 165 Received 17 Jan. 1980 ISSN 0001-6772. Department of Medicine, G-I Unit, Karolinska Hospital, Stockholm, Sweden.

The output of bile and pancreatic enzymes after two mixed meals with different fat content was studied in 16 healthy volunteers of normal body-weight. A quantitativ multiple indicator dilution method was used. The delivery of more fat per time to the duodenum after the fat rich compared to the low fat meal was followed by the emptying of mor bile but did not significantly increase the pancreatic response The ratio between amylase and lipase was constant throughout the course of single experiment but differed between the subjects The ratio decreased with an increasing body weight, indicating a relative dominance of amylase over lipase in the lighter and the reverse in the heavy subjects. The same correlation was found when results from a previously reported series with a glucose containing mixed meal were reexamined. It is proposed that long time dietary differences might influence the enzyme composition of the postprandial pancreatic secretion

Key ords Bilirubin excretion, gastric emptying, indicator dilution technique pancreatic secretion, test meal

es of the composition of a mixed meal alter livery of nutrients to the duodenum and thus mulation to intestinal functions (Lagerlof sson & Ekelund 1976) In a previous study it nown that the addition of more protein to a meal increases the gastric emptying rate of a and the secretion rat of pancreatic nes whereas the gallbladder emptying is unaf l (Ekelund & Johansson 1975). This study re- quantitative measurements of the biliary and eatic response to mixed liquid meals with dif t fat content in healthy non-obese subjects.

HODS

ial Two 300 ml testmeals differing in their content ere administered to 16 normal healthy volunteers, m and 3 omen aged 20 to 38 years (Table 1). med consent as obtained from each subject and the was approved by the Ethical Committee at the ska Institute.

rimental procedure The multiple indicator dilution ique requires intubation of the gastrointestinal tract with 3 polyvinyl tubes of different lengths. The set of 3 tubes is swallowed in the afternoon before the experimental day and are allowed to pass down the gastrointestinal tract by normal peristalsis After an overnight fast the position of the tubes is checked by fluoroscopy The tube tips should be positioned in the gastric antrum, in the duodenum descendens and in the jejunum 75 cm from the antral tube The gastric residuum is removed before starting the experiment and salivary secretion is continuously withdrawn.

From the time of meal intake and on, the intestinal

Table 1 *Composition of t st me ls (g/100 ml)*

	NO low fat meal	NOF high fat meal
Skim milk protein	3.6	3 6
Lactose	5 1	5 1
Corn oil	5 9	11.8
Polyethylene glycol (PEG)	3.0	3.0
Number of subjects (females)	9 (2)	7 (1)
Body weight (M ± S D)	75±13	74±14

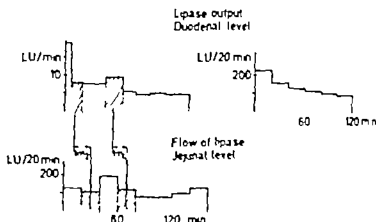


Fig. 1. Time relationship between the amount of lipase passing the jejunal sampling site and the rate of lipase output at the duodenal level expressed as LU/min or, to facilitate comparison between different experiments, as LU/20 min.

content are continuously marked with 3 different indicators infused through the duodenal tube at a constant rate (0.5 ml/min). The indicators unlabelled vitamin B, ?Co-B and ?Co-B are sequentially infused with change of indicator every 40th minute.

Gastric samples are withdrawn at 20 min intervals. The jejunal contents are continuously siphoned by 20 min collections, and are kept at −20° until analysed.

Chemical method. Polyethylene glycol was determined according to Hydén (1955). Protein was determined with Reiders modification of the method by Folin-Ciocalteau (1959) and fat by titration of free fatty acids after complete hydrolysis. Carbohydrates was determined by the Hagedorn method (1935). Bilirubin was measured according to Jendrassik & Groof (1938), lipase according to Desnuelle, Constantin & Baldy (1955) and amylase according to Lagerlöf's modification of Nörby's method (1935).

Calculation. A detailed description of the calculations has been given elsewhere (Lagerlöf et al. 1976). The duodenal indicators mix with the emptied gastric marker (PEG) during transit of the segment. The concentration of the mixed indicators in the jejunal collections are used to calculate flow rates of chyme at the jejunal sampling site (ml/20 min). By adjusting the scale of each indicator to the same magnitude the sequential infusions of 3 different duodenal indicators can be regarded as the infusion of one marker at a constant rate. The difference between the rates at which the indicator is infused into the duodenum and passes the exit of the test segment equals the change of transit time and the addition of consecutive changes expresses the varying transit times in the segment. The transit time is used to compensate for the time lag between the passage of test meal marker, pancreatic enzymes and bile at the jejunal level and their entrance into the duodenum. The output of bile and pancreatic enzymes are thereby adapted to the level of the Vater's sphincter (Fig. 1) and the gastric emptying of marker and content can be assessed. The emptying of the different meal components have to be calculated separately due to intragastric separation. The calculations are considered correct if the emptied amount equals the amount in the test meal.

Statistical methods. Results are given as the mean ± standard error of the mean (M ± S.E.). Significance was tested by the Wilcoxon test for ... samples and ... by the Spearman rank test.

Linear regressions were calculated between ... of amylase to lipase with time.

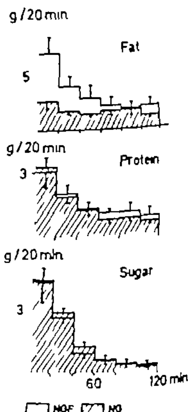


Fig. The gastric emptying of different food com... (M ± S.E.M. g/20 min) after the high fat meal (NOF) ... the low fat meal (NO).

Correlation betw ee body w eight and gas- tying during the first hou aft intake of fat meal

Body weight kg	Gastric emptying Energy (kJ 0–60 min)	Fat (g 0–60 min)
62	478	8.7
64	561	8 7
65	704	14 5
71	577	11 1
85	1 341	17.2
87		
100	985	26 5
	<0 05	<0 05

TS

emptying More fat was emptied to the um during the first 40 min after the high ted with the low fat meal (11 +1 7 g vs. 7 g, *P* 0 01) The emptying of protein and as not affected by the added fat (Fig.) A e correlation was previously reported be the body weight and the early emptying of eal energy from the low fat meal (Johansson A similar correlation was found also after the h meal (Table 2)

acid loads to the duodenum did not differ n the meals, an average 22 6±4 0 and 1 2 mmol hydrochloric acid being emptied af low and high fat meal respectively

ut of bile After an initial gallbladder empty output of bilirubin is approximately constant 0 min and on (Fig 3) More bile was emptied the first 40 min after the fat rich meal com with the meal with a lower content of fat 05)

put of pancre ic nzyme The output rates mylase and lipase ere not significantly ted by the added fat (Fig 3) and the total reatic response was not significantly different een the meals

parallel secretion of the pancreatic enzymes ghout the course of each experiment is indi d by the absence of any correlation between the amylase to lipase and time The average b-val were 0 0029 and −0 0063 for the low and high meal respectively

correlation was found between the body weight

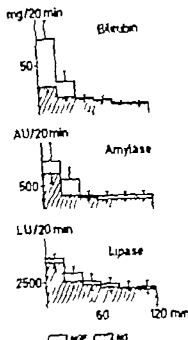


Fig 3 The output of bilirubin (M±S E.M. mg/20 min) amylase (M±S.E.M AU/20 min) and lipase (M S.E M LU/20 min) at the level of papilla Vateri after the high fat meal (NOF) and the low fat meal (NO)

and the pancreatic enzyme composition The more heavy the subject the lower was the ratio between amylase and lipase (Table 3) In the high fat series the total output of amylase decreased at an increasing body weight

DISCUSSION

The gastric emptying of liquid nonhypertonic meals is rapid in the beginning then slowing to become almost constant (Johansson 1974) To this corre sponds an initial rapid intestinal transit of the meal followed by a slower oscillatory ntestinal propagation (Johansson 1974 Lagerlöf et al. 1976) Knowledge of the variations in transit times is prerequisite to adjust the jejunal flow of bile and enzymes to the output rate at the level of the Vateric sphincter The enzyme output at this level follows the pattern of gastric emptying The distribution of enzymes then changes along their transit along an intestinal segment and the postprandial enzyme profile is thus different intestinal levels. This may be one reason which beside differences in duodenal stimulation

Table 3 *Correlation between body weight and the total output of lipase and amylase after the meal*

Subjects 1 and 4 were females

Exp No	Body weight (kg)	Broca's index x)	Amylase (AU 10³)	Lipase (LU 10³)	AU/LU
1	62	0 86	7.5	12 3	0 61
2	64	0.85	1 7	12 7	0 14
3	65	0 87	5.5	3 8	0 23
4	71	1 01	2.4	9.3	0 76
5	85	1 08	0	22 7	0 09
6	87	0 97	1 3	13 4	0 10
7	100	1 04	0 7	15 0	0 05
P			<0 05	>0 05	<0 05

Broca's index body $\frac{\text{body weight}}{\text{length} - 100}$

explains the different secretory patterns of pancreatic enzymes reported in literature (Brunner et al 1974 Ekelund & Johansson 1975 Hong Chin & Hur 1961) The delivery of more fat per time unit to the duodenum after the fat rich meal was followed by the emptying of more bile whereas the pancreatic enzyme response was not significantly increased This supports the suggestion of a lower maximal threshold for pancreatic secretion than for *gallbladder contraction (Brunner et al 1974)*

The parallell secretion of the different enzymes during the course of each single meal is in agreement with the results of Hong et al (1961) Not reported before is the relation between body weight and the enzyme composition of the pancreatic secretion The ratio between amylase and lipase was found to decrease at an increasing body weight due to a lowering of the amylase content Differences in enzyme inactivation due to different transit times cannot explain these results Heavy subjects do have shorter transit times than lighter (Johansson & Ekelund 1976) but variations are less marked between the subjects than during the course of a meal in one subject and the ratio amylase to lipase was constant throughout each experiment The intestinal loads of hydrochloric acid were not different in heavy and light subjects

The present data are however insufficient to exclude the possibility that the early emptying of more fat and energy in heavy subjects rather than the body weight determines the pancreatic enzyme composition Previously reported experiments with a hypertonic mixed meal (Lagerlöf et al 1976) were therefore reexamined This meal contai[ned] instead of fat as the dominant energy early emptying is unrelated to the bod[y] Also after this meal the ratio between am[ylase] lipase was negatively correlated to the bod[y] (Fig 4) and there existed a positive correl[ation] tween the body weight and the total lipa[se] (r+0 6217 P<0 02, n 15)

The interrelationship between the bod[y] and the pancreatic enzyme composition [in]terpreted in terms of a long time adaptat[ion] pancreatic gland to differences in food int[ake] higher the caloric density of a meal the larg[er] amount of energy delivered to the duoden[um] after meal intake as demonstrated from

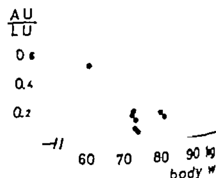


Fig 4 The bodyweight is negatively correlated [to the] ratio between the total amount of amylase and l[ipase] AU/LU (r−0 6863 P<0 01 in 15 healthy subjects (n[or]mal bodyweight (two females) after a 300 ml meal had the same composition as the low fat for the addition of 10 g glucose per 100 ml

1 by Hunt & Stubbs (1975) and own studies son 1976, unpublished data). Hunt & Cash id further found that heavy people prefer th higher energy density than to those of or less bodyweight. Different dietary habits and light subjects should thus imply differ rees of stimulation of the pancreatic gland ng periods of time. Evidence for long term nfluence on the enzyme composition in s been presented by *Dreiling et al.* (1962) und a low amylase content in pancreatic om obese patients, which was almost dou- er 6 months of dietary restriction.

...ier studies have to be made to define causal tions between body weight, dietary habits ncreatic enzyme composition. A direct con- ce of the body weight-related ariations in atic enzyme composition is that clinical ations of the exocrine pancreatic function include determinations of at least two ...s.

...k M. Andersson, I. Bergstrom, L. Kostic, M.-L. ...mglon, L. Nordberg, S. Ritterwall and R. Östman ...able assistance. This investigation was supported ...ds from the Medical Research Council (B77-19X ...1) and from the Karolinska Institutet.

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ges in cerebral blood flow during hyperventilation 'O₂-breathing measured transcutaneously in humans bidirectional pulsed ultrasound doppler d velocitymeter

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HAUGE, A. THORESEN M & WALLØE, L. Changes in cerebral blood flow during hyperventilation and CO_2-breathing measured transcutaneously in humans by bidirectional pulsed, ultrasound doppler blood velocitymeter Acta Physiol Scand 1980 110 167–173 Received 20 Jan. 1980 ISSN 0001-6772. Institute of Physiology University of Oslo Norway

W have used bidirectional pulsed ultrasound doppler ystem which measures the instantaneous mean elocity acros the lumen of a blood vessel in order to determine the relationship between alveolar P_{CO_2} (P_{ACO_2}) and blood flow in the four arteries supplying the brain in humans. Both high and low P_{ACO_2}-values were explored Six subjects, 3 males and 3 females (22–40 years) were studied by use of this non-invasive technique To increase the P_{ACO_2} the subjects were breathing 4 6 and 8% CO_2 in air P_{ACO_2} was reduced by voluntary hyperventilation down to chosen end-expiratory P_{CO} value of about 2.2 kPa We found linear relationship between arterial blood flow xpressed as percentage of control level and P_{ACO_2} in the range from 3 3 to 7.3 kPa. At the very lowest P_{ACO_2} values levelling off of the response with flow values of 40 to 45% was observed The CO_2-reactivities in the 6 persons aried between 28.1 and 30 0%/kPa. The time course and the magnitude of the flow response were similar in all four arteries.

lifferent method have been used to measure l blood flow in the experimental animal. few of these methods can be applied to ›, the most important today being the elec netic, the Xe-injection and the Xe-inhalation ls The electromagnetic flowmeter (review ference Mills 1972) can measure the in- neous blood flow through a cross section of the internal carotid or vertebral arteries. The isadvantage of this method is of course that it 's surgically exposed artery with the patient general anesthesia It is therefore only applied mans during certain surgical operations, and nly on quite specific indications. The ^{133}Xe terial injection method is in much more fre use on patient (e g. Tomunaga et al 1976 et al. 1977 Hachinski et al. 1977). This d does not measure the instantaneous blood only the average flow over some minutes. The ; of uncertainty in each single measurement is considerable and the risk connected with the carotid or femoral puncture and the discomfort of the patient seriously limit the number of measurements one can obtain from each person In practice there is a time span of at least 20 min between each injection. The method does give information about the regional distribution of flow (Olesen et al. 1971 Ingvar & Lassen 1977). However it cannot be applied to the vertebral artery During the last few years the ^{133}Xe inhalation method has been improved (Obrist et al. 1975 Meyer 1978) This method is noninvasive but the clearance curves are contaminated by radioactivity from the scalp and other extracranial sources

A fourth method which potentially could be useful on patients is the ultrasound doppler method. In their earliest papers Baker Franklin Rushmer and collaborators (1959 1961) indicated the possibility of developing equipment which could measure volume flow Due to practical difficulties and to the

operating principles implemented in the instruments available commercially these instruments have not yet been able to do this

In our laboratories we have developed a bidirectional pulsed ultrasound doppler system ("UNIDOP") which measures the instantaneous mean velocity across the lumen of a blood vessel (Hatteland & Eriksen 1980 Wille 1977) As long as the cross sectional area of the vessel is constant this mean velocity is proportional to flow The performance of the instrument used transcutaneously has been compared to simultaneous electromagnetic flowmeasurements on the same common carotid and femoral arteries in dogs (Guldvog et al 1980) The similarity in flow pulse shape was good

As a result of these encouraging observations we wanted to explore the possibility of using the instrument to measure cerebral blood flow in humans For our first experimental series we wanted to choose a physiological situation where the flow response to a certain degree is known from the use of other methods We have chosen to use the flow response to step changes in alveolar P

The relationship between alveolar P (P) alternatively arterial P (Pa) and cerebral blood flow has been investigated by many authors both in animals (e g Reivich 1964 Harper & Glass 1965 Grubb et al 1977) and in humans (Kety & Schmidt 1946 1948 Severinghaus & Lassen 1967 Tominaga et al 1976) In most experimental animals the blood flow to the brain is a sigmoid function of P_A with normal resting P values on the steepest part of the curve The relationship in humans is of course not explored to the extreme P_A values and there is large scatter in the individual points obtained by the Xe injection method In spite of these difficulties most authors describe a sigmoid relationship also in humans (Kety & Schmidt 1948 Olesen et al 1971 Tominaga et al 1976) with a linear relationship in the neighbourhood of normal resting values Our results obtained noninvasivly are close to the results obtained by previous authors the main difference is a more extended linear part in the middle of the curve

A preliminary report on some of the results has been given at a meeting of The Physiological Society (Thoresen & Walloe 1979)

METHODS

The doppler velocitymeter UNIDOP used in the present investigation and the computer systems used for data analysis have been described in detail elsewhere (Hatteland & Eriksen 1980 Wille 1977)

UNIDOP operates on two different frequencies 1.5 MHz or 6 MHz and can operate either in a continuous wave mode or in a pulsed wave mode on either frequencies. The low frequency 1.5 MHz is used for measuring velocities in deep vessels and in this mode the depth range is from 1 to 10 cm The 6 MHz frequency is used on more superficial vessels with diameter [illegible] mm and the depth range is from 0 2 to 5 cm

UNIDOP contains sharp low-pass filters (Butterworth) adjustable to the four different frequencies Depending on the measurement and velocities expected one can choose among the (200 400 and 700 Hz) These filters [illegible] caused by vessel wall movements and [illegible] bances

The velocitymeter is connected to an analog time-compression spectrum analyzer [illegible] the doppler signal is fed through 63 band pass [illegible] 20 ms giving the relative intensity of all the [illegible] present in the vessel at that point in time It is [illegible] to see the spectrum on a scope simultaneously with the audio signal from the loudspeaker One use of the time spectrum is to adjust the amplitude [illegible] eliminate most of the noise without losing too [illegible] information-containing signal Another use of the [illegible] trum is to discriminate between different [illegible] ing on the characteristic shape of their spectra

To store the audiosignal for later calculations [illegible] DOP is connected to a stereo casette taperecorder (GXC 760 D) The doppler signal is recorded on [illegible] nel and the ECG and comments are recorded [illegible] channel Both the tape-recorder and the velocity[illegible] connected on line to a NORD computer system [illegible] tical reasons we usually store the recordings on [illegible] tape and play the tape back for later calculation [illegible] computer The computer calculates the spectra [illegible] ms using a fast fourier transform program and [illegible] mean velocity from each spectrum (Wille 1977)

The whole cross section of the vessel is [illegible] ments illuminated by a soundfield of approximately [illegible] stant intensity (Guldvog et al 1980) If one [illegible] the cross sectional area of the vessel does not [illegible] during the recording changes in the mean velocity [illegible] proportional to changes in instantaneous [illegible] The computer also calculates the time average [illegible] instantaneous mean velocity in one cardiac [illegible] triggered by the ECG This average mean [illegible] proportional to the average volume flow during the [illegible] diac cycle

The experiments reported in this paper were performed on six subjects 3 males and 3 females (22–40 years) [illegible] were normotensive and without any known [illegible] ease During the experiment the subject was [illegible] quiet room with temperature 22–25°C with a [illegible] and breathing through a nonrebreathing [illegible] nected to a suitable mouthpiece

To increase the P_{CO_2} the subjects were breathing 8% CO_2 in air for about 4 minutes To lower [illegible] were hyperventilating voluntarily down to a [illegible] sen end-expiratory P_{CO_2} value During the hyper[illegible]

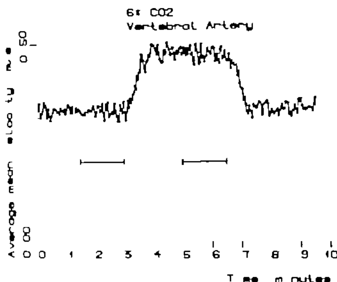


Fig 1 A erage blood elocity in the right ertebral artery during CO breathing The figure show the recording of blood velocities in the right vertebral artery Each point represents the time average over one cardiac cycle of the cross-sectional mean velocity in the vessel The figure displays the values for each third cardiac cycle As long as the cross-sectional area of the vessel is constant, the average elocity is proportional to flow (see Methods) The right ertebral artery   as measured on   26 year old male subject with   1.5 MHz transducer in 4 cm depth behind the mastoid proces   of the skull Mean velocity in the marked out 1.5 min period prior to the breathing of gas was 0 3295 m/s, SD   as 0 0121 m/s, giving   CV = 0 0369 The corresponding   alues during gas breathing   as 0 4740 m/s, 0 0144 m/s, and 0 0340 Thus, in this   xperiment the flow increased to 144 % of the resting value during gas breathing P_{A,CO_2} increased from 5 48 to 6 97 kPa.

periment, the end-expiratory P_{CO_2} values were con- ...tly read aloud to the subjects, and they tried to ...their breathing to keep   certain P_{A,CO_2} hich they ...ed   ithin   2% of this value End tidal P_{CO_2} as ...uously monitored with an infrared CO   analyzer ...man LB-2, connected to   Hewlett-Packard poly- ...)

...flow measurement on the internal carotid artery ...MHz transducer was held with approximately 45° to the direction of the artery beneath the angle of the ...ble depth varying between 1 0 and 1 7 cm Measuring ...ts vertebral artery the 1 5 MHz transducer was held ...d the mastoid process pointing to the bottom of the ...ate orbita to catch the artery between atlas and oc... The depth varied between 3.5 and 5 cm. The angle ...ween the transducer and the artery is however un...n, because of the large individual variations in the ...se of the artery (Lizza 1974).

...ce CO_2 breathing only causes   slight hypertension ...than change in blood pressure per se does not affect ...bral blood flow (Tomonaga et al 1976), we have not ...sured the blood pressure systematically during the ...riments

RESULTS

I   one series of experiments the content of CO_2 in the inspired air was increased in one step from 0 to 4 6 or 8% and the resulting blood velocities in one of the internal carotid or vertebral arteries were measured. Fig 1 shows the results from one such experiment. As illustrated in the figure the blood velocities in a steady state situation only vary within narrow limits. Typically the coefficient of variation is less than 0 05 As the gas mixture is switched on, the blood velocities increase through a short transient period to a new steady state The magnitude of the increase is larger with higher CO_2 content in the air When the gas mixture is switched off the blood velocities return to resting values within one minute

The time course and the magnitude of the flow response were similar in all four arteries supplying

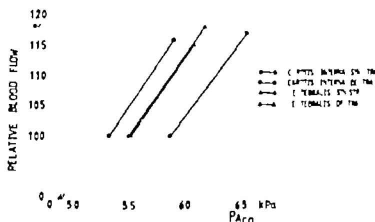


Fig 2 Relative blood velocity in each of the four vessels supplying the brain as function of P Abscissa Average P in two steady state periods of 1.5 min duration approximately min apart one immediately before breathing 4% CO in air and one during such breathing in four different experiments Ordinate Percentage change in blood flow in each of the four vessel supplying the brain in one subject due to the breathing of 4% CO The four vessels were measured on four different days in the same object as in Fig 1 The CO reactivity in these four vessels varied between 22.5 and 28.5%/kPa (3 4 and 3 8%/mmHg)

the brain Fig. 2 displays the results from four experiments on one person one from each artery Although the figure reveals large differences in resting P_A explained by the fact that the experiments were performed on four different days the

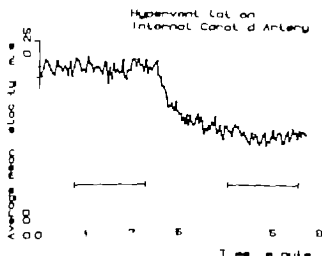


Fig 3 Blood flow to the brain during hyperventilation The tracing shows the blood velocity in the right internal carotid artery during a hyperventilation experiment The measurements were obtained with a 6 MHz transducer in 1.5 cm depth beneath the angle of the jaw on the same subject as in Figs 1 and 2 Mean velocity in the 1.5 min period prior to hyperventilation was 0.2127 m/s and CV was 0 0338 The corresponding values during steady state hyperventilation were 0 1198 m/s and 0 0576 P decreased from 5 6 kPa to 3 9 kPa

CO_2-reactivities (that is the slopes of the four e g % flow change per CO_2 pressure unit) are similar It was always found in our experiments on normal subjects that the four arteries supplying the brain had equal CO_2-reactivity

In another series of experiments the P_A was lowered by voluntary hyperventilation down to a certain preset P value Fig 3 shows a recording from a typical experiment on the right internal carotid artery In this experiment the blood flow displayed an initial sudden decrease followed by a slower decrease The detailed time course in the experiments depended on how the subject and on the pre-determined P_A value

Fig 4 displays the steady state values of from all experiments performed on one subject both during CO_2-breathing and during hyperventilation To eliminate the variation from one experiment to another in resting P_{A,CO_2} we have added the difference between 5 33 kPa and the measured resting P both to the high and the low P_{A,CO_2} values in each experiment The resulting normalized relationship between P and cerebral blood flow is shown in Fig 5 A There is a linear relationship in the P_A range from 3 3 to 7.3 kPa (25 to mmHg) At very low P_A values (less than approximately 3 0 kPa) the figure suggests a levelling off of the flow response with relative flow values of the order 40 to 45%

The results from the other persons investigated

Fig. 4. Relative blood flow as function of P_{ACO_2}. Abscissa and ordinate as in Fig. . The figure displays the results from all experiments performed on one person. The results shown in Figs. 1, 2 and 3 can also be found in this figure.

similar. Altogether six persons have been ated in comparable detail. They all dis- linear relationship between P_{A} and flow. $)_2$ reactivity was equal for all six, well within ement accuracy. Fig. 5B, C and D show blood flow as function of P_{A} for three of e persons which has not been used in the us figures. The four panels in Fig. 5 illus- the typical individual differences. At low values there were larger variations in relative flow than in other parts of the curves due to curate measurements when the subjects were entilating violently and probably also t m- al differences.

USSION

esults presented in this paper show a simple nship between P_{ACO_2} and average blood vel- in all four arteries supplying the brain. The nship is linear over most of the investigated -range only levelling off for very low values. Not only are the results highly repro- ible for each person, but the results are also similar for all six persons investigated, with shape and equal slope of the straight lines. CO_2-reactivities found in our 6 persons varied een 28.1 and 30.0 %/kPa (3.75–4.1 %/mmHg) a median of 29.4 %/kPa (3.9 %/mmHg).

e conversion from average blood velocity to relative blood flow rests on the assumption of a constant cross-sectional area of the artery through- out all measurements made on that artery. This assumption is of course questionable. Changes in the cross-sectional area of a vessel could be caused either by changes in blood pressure or by changes in tonus of the smooth muscle in the vessel wall. Changes in area caused by changes in blood pres- sure are probably not important in our experiments. This conclusion is valid both for the area changes in each cardiac cycle, which is less than 6 % in humans (Olson 1974, Schabert 1978) and for long term changes, since blood pressure shows only minor variations with changing P_{A} (Tominaga et al. 1976, Olesen et al. 1971, Kety & Schmidt 1948). Less is known about the effect of changes in P_{ACO_2} on the smooth muscle in the wall of the arteries. While we tend to believe that also this effect must be small, we have only indirect evidence: (1) There are no reports in the literature of effects of changes in P_{CO_2} on the tension of isolated strips of vascular smooth muscle, while such effects of changes in P_{O_2} are well known (2). The CO_2-reactivity values found in our experiments are in good agreement with reactivity values found by others. Fig. 3 in Kety & Schmidt (1948) shows a CO_2-reactivity in the neighbourhood of 3.75 % mmHg around resting values and an increasing reactivity with higher P_{ACO}-values. They used the N_2O inhalation method. Tominaga et al. (1976) using the Xe injec

A

B

C

D

Fig 5 Relative blood flow as function of normalized P   in four individuals. The resting P_{ACO_2} values b normalized to 5 33 kPa (=40 mmHg) by adding or subtracting the pert nent figures. (A) Thi figure contains t result as Fig 4 (male 26 years old) (B) Female __ years old (C) Male 27 years old (D) Male 40 years old

tion method present a semilogarithmic relationship between P $_{O_2}$ and cerebral blood flow with a CO reactivity of 6 4/mmHg near resting values Olesen et al (1971) using the same method present a CO_2 reactivity of 4% (4%±1 1%) and a sigmoid relationship between CBF and P_A It is difficult to explain the close agreement between our results and the results obtained by others without assuming a constant cross sectional area of the arteries We would however like to have some more direct measurements on the cross sectional area of the vessels For the internal carotid artery such measurements could be obtained by a high frequency ultrasound echo technique and we intend to extend the experiments in this direction in the future

The uncertainty in the determination of the true mean cross sectional blood velocity caused by uncertainties in the angle between the velocity vector and the sound-beam is probably not large internal carotid artery However the verte tery has a very complicated and twisted c the suboccipetal region where we are me causing uncertainties concerning the ang probably explains the large variations fo tween the different persons in resting bloo ties in the vertebral arteries In contrast similar velocity values found in the internal arteries

If our assumption of a constant cross area of the arteries turns out to be valid trasound doppl r technique seems to be su other methods This is especially true if it to be possible to estimate the cross sectiona the vessel and thus the absolute values (Guldvog et al 1980) and not only relative in flow

...ike to thank Morten Kjærnes for help with the ...ta, and Knut Liestøl, Arild Njå and Jan Mæhlen ...ir comments on the manuscript. M. T. was ...by ... student fellowship from The Norwegian Council for Science and the Humanities. Finan... ...rt was also provided by The Royal Norwegian ...for Scientific and Industrial Research and by Vara...

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noregulatory responses to tyramine in the pigeon

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HISSA R GEORGE, J C STEVENS E. D & SAARELA S Thermoregulatory responses to tyramine in the pigeon. Acta Physiol Scand 1980, 110 175–180 Received 21 Jan. 1980. ISSN 0001-6772. Department of Zoology Zoophysiological Laboratory University of Oulu Finland and Department of Zoology University of Guelph, Guelph, Ontario, Canada.

Intravenous injections of different doses of tyramine induced hypothermia in the pigeon in the cold and moderate hyperthermia in the warm environment The hypothermia was correlated with dose-dependent decline in shivering. Hyperthermia was attributed to the chrono- and inotropic effects of tyramine The indirect stimulatory effect of noradrenaline (NA) at the tissue level were studied Pretreatment with α-methyl-para-tyrosine (α MpT) and blocking α-adrenoceptors with phentolamine diminished the hypothermia induced by tyramine in the cold The results obtained indicate that the release of endogenous NA stimulated by tyramine might result either in hypothermia or hyperthermia, thus resembling similar effects obtained with exogenous NA in birds.

Key word Tyramine, noradrenaline alpha-methyl-para-tyrosine phentolamine hypothermia, hyperthermia, electromyographic activity

ttle information is vailable on the effects of ne on thermoregulation in birds. Key & Mar 67) observed that although neither i nor inistration of tyramine (100–250 μg per) had any effect on behavioural and elec ical activity in 1 28 day old chicks yet i t produced behavioural and electrocortical L El-Halawani et al. (1970) noted in 5 and 9 old cold-adapted chicken that i injection amine (200 mg/kg) decreased both xygen nption (VO_2) and body temperature but ined electromyographic activity

as been suggested that tyramine releases the enous noradrenaline (NA) from its storage es in nerve endings Since the liberated atter is subjected to intraneuronal de tion before reaching the extraneuronal space Trendelenburg 1972 Brandão et al. 1978) a very small amount of NA escapes de tion Nevertheless, tyramine has repeated- n show to produce sympathomimetic effects by its action in preventing the reuptake of ed neurotransmitters (see Trendelenburg

lha-methyl-para-tyrosine (α-MpT) is a potent competetive inhibitor of tyrosine hydroxylase enzyme (see Nagatsu et al 1964 Udenfriend et al 1965) and it is widely used to suppress the endogenous catecholamine synthesis (e g. Spector et al 1965 Saarela et al. 1977 Widerlov 1979). In our previous study Saarela et al 1977) α-MpT was shown to abate significantly catecholamine synthesis in the temperature stressed pigeons 4 h after the administration

The present investigation was undertaken in order to study the effect of endogenous NA on body (T_b) and foot (T_f) temperatures electromyographic (EMG) activity heart rate and blood pressure at three different ambient (T_a) temperatures after the stimulation of its release with i tyramine In addition, the effects of tyramine after blocking catecholamine synthesis with α MpT and blocking α-adrenoceptors with phentolamine were examined

MATERIALS AND METHODS

Adult pigeons of both sexes weighing 330–395 g, were obtained from commercial supplier and were acclimated in individual cages in thermostatically controlled room at 22°C at least for period of one month prior to the com-

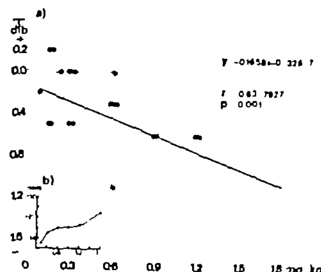


Fig 1a Dose response of the mean maximal fall in body temperature (T_b) to i v injection of different doses of tyramine in the cold The linear regression is fitted to all points by the method of least squares Altogether 44 expts were performed with ... pigeons.
Fig 1b Mean time to maximal fall in T after different dose of tyramine (mg/kg)

mencement of experiments The lighting regimen was 12 h of light and 1 h of darkness Relative humidity was maintained at approximately 30 %

All measurements were performed in a darkened chamber maintained in a regulated water bath either at 4 4° or 30°C as described by Hissa et al (1980*a*)

The pigeons were kept unrestrained in the chamber and were given 1– h for adjustment before injections All injections were made between 11 00 and 13 00 hours

For measuring cloacal and food temperatures shivering and heart rate the methods have earlier been described by Hissa et al (1980*a*) Briefly the temperatures were recorded continuously throughout the expt on a potentiometer and displayed with a Gra s penrecorder Muscle potentials were recorded from pin electrodes inserted into the breast muscle Shivering was measured as described earlier by Hissa et al (1980*b*) by integrat ng electromyograms (EMG) and feeding the amplified and rectified po-

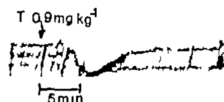


Fig 2 A typical tracing of EMG integrals after ...tion of tyramine (0 9 mg/kg) in the cold

tentials (time constant 0.04 s) with a Grass 7P... 7P10 B Integrator triggered to reset every ... height of the record reflected the total shivering ... put was displayed with a Grass penrecorder

For i v injection of tyramine or saline ... technique as described by Hissa et al (1980a) ... For intrahypothalamic injections of tyramine ... guide cannula was implanted stereotactically ... terior hypothalamus as described earlier (... Rautenberg 1974). The modified stereotaxic co... (Karten & Hodos 1967 Pyörnila et al 1977) we... mm anterior from the intra-aural-line 1 0 mm la... the mid-line and 9 0-10 0 mm below the surface ... varium

The direct blood pressure was recorded con... by cannulation of the brachial artery and the ... Physiotransducer (Alltech MSD 10-5). The output ... transducer was displayed with a Rikadenki DP... shin) recorder Tyramine was administered (i v ... tibial vein ECG was recorded with a Phy... (Schwarzer Varioscript V8..2) and EMG was ... by the electrodes fed into the differential preamp ... a band-pass filter The high and low-cut (3 dB ... cies of the filter were 500 Hz and 10 Hz respective... amplified and filtered signals were rectified and ... with a Rikadenki DP-6 recorder

In studying the effect of i v tyramine (1 mg/kg ... of injection at 20°C on the endogenous pl... catecholamines plasma adrenaline (A) and nor... (NA) levels were assayed spectrophotofluorom... according to the method of Diamant & Byers (... modified by Hohtola et al (1978).

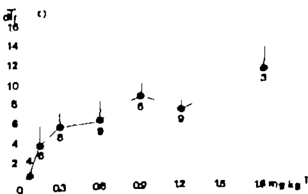


Fig 1c Dose-response curve of T (i v tyramine in pigeons at 4°C Vertical bars indicate ± S E of mean Number of animals used are given at each point

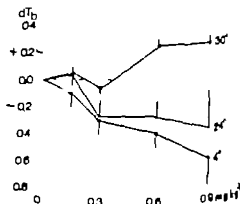
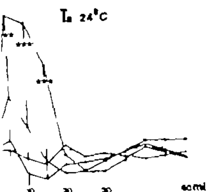


Fig 3 Compa sons of changes in T after different ... of tyramine 4°C (●—●) 24°C (▲—▲) and 30°C ... in the pigeon Each symbol represents 4–6 separate ... urements. Vertical line indicate ± S E of mean



...he time-course of changes in heart-rate (dH,) of ... to 0 1 (Δ—Δ, N 4), 0.3 (O—O N=4) 0 6 ... N 5) and 0 9 (●—● N 5) mg/kg of ... at 4°C. Values indicate positive and negative ... in comparison to its preinjection values. Bars ... ± S E of mean *** $P<0.001$

...Tyramine HCl (Sigma) and noradrenaline (NA ...nol bitartrate, Sigma), Phentolamine Mesylate ...itine, CIBA) and d,l-alpha-methyl-paratyrosine ... Sigma) were dissolved in 0 85% w/ NaCl (saline) freshly every day Control pigeons ... the same volume (0.1-0 3 ml or 1 μl) of saline ... or intrahypothalamic injections All doses of ... are expressed in terms of base

...is obtained are expressed as the mean ± S.E of ... statistical analysis was performed with Student' ... paired comparisons. Regression line and cor... coefficient were calculated according to the ... of least squares Results occurring with change ...ity of less than 0 05 were considered statistically ...nt

...LTS

...ral injections

... and show the effect of ... injections of ... at doses of tyramine on T and T in the cold ... response curve was obtained by recording the ... tion fall in cloacal temperature for each ... and described by the method of least ... At this T_a, the mean T was 39.6±0 1 ... and T 13.2±0 9°C ($N=77$) at the beginning ... expts Saline injection had no significant ef... the level of the variables measured The ... T is correlated with an increased heat loss ... icated by the elevated T following the injec... and is correlated with decrease in heat produc... as indicated by the decreased EMG of shiver... Note that a significant depression of T (mean

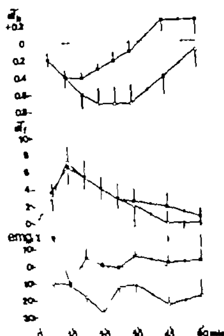


Fig 5 Time course responses of the body temperature expressed as mean maximal changes (dT_b, °C), foot temperature (dT °C) and electromyographic activity (emg in percentage) to tyramine 1... mg/kg in the pigeon pretreated with α-methyl-para-tyrosine (250 mg/kg) either ... h (□—□) or 4 h (■—■) before tyramine injection at 4°C Each point is the mean maximal change over the preinjection value Vertical bars indicate ± S E. of mean. $P<0.05$

−0.26°C) was observed at a dose of only 0.3 mg/kg ($P<0.001$) and that the most dramatic effects of T also occurred at low dose levels (T increased 5°C at a dose of 0.3 mg/kg) The pattern of integrated electromyographic activity after a single i v injection of 0 9 mg/kg tyramine is shown in Fig. 2 In general the first sign of the decline in shivering did not occur earlier than 3–5 min of the injection. As shown in Fig. 3 the thermoregulatory response to tyramine depends on the ambient temperature At 30°C the highest doses (0 6–0.9 mg/kg) caused a moderate rise in T_b. At 24°C a slight fall in T was observed with 0.3–0 9 mg/kg of tyramine (not dose-dependent) However at 4°C a clearly dose dependent drop in T was substantiated.

In analysing the possible causes for an elevated T in the warm environment in response to tyramine the chrono- and inotropic effects at 30°C and 24°C, must be considered. The time course of the changes in heart rate (beats/min) at 24°C are

Table 1 *Effect of i v injection of tyramine (1.2 mg/kg) after pretreatment with α-methyl-p... (α-MpT) and phentolamine (Ph) on the mean maximal body (T_b) and foot (T_f) temperature change*

Pretreatment Time Dose	Saline	N	α MpT 2 h 250 mg/kg	N	α MpT 4 h 250 mg/kg	N	Ph 30 min 3 mg/kg	N
dT_b	−0 7±0 13	6	−0 7±0 19	6	−0 4±0 07	6	−0 ±0 05	
dT_f	+7 ±0 97	9	+6.. ±1.83	6	+7 ± 08	6	+3 5±2.52	

4 h α MpT vs saline not significant Ph vs saline $P<0\ 01$

illustrated in Fig 4 As depicted tyramine produced dose related effects on heart rate Significance values are given in the figure With the greatest dose (0 9 mg/kg) the elevation of heart rate was during the first 5 and 10 min (135±14 and 128±17 beats/min respectively above the preinjection level of 162±17 beats/min) Quantitatively and qualitatively corresponding elevation in the heart rate from 133±8 beats/min to 284±64 beats/min was attained at 30°C too

Pretreatment of the pigeon with α MpT (250 mg/kg) 2 or 4 h before tyramine injection reduced the fall in T_b only after 4 h of the tyramine injection (Fig 5 Table 1) No differences in foot temperature were obtained The normally produced decline in shivering with tyramine (0 9 mg/kg) was markedly reduced after pretreating the animals with α MpT 4 h before tyramine administration (Fig. 5)

As shown in Table 1 phentolamine (3 mg/kg) caused a significant antagonism to tyramine ($P<0\ 1$)

To study the effect of tyramine on the blood pressure 3 dose runs of 0 8 1 6 and 3 2 mg/kg at 10°C were performed With the two smaller doses the systemic blood pressure was elevated by only 8–14 mmHg whereas the 3 2 mg/kg dose of tyramine was sufficient to cause an increase of 55 mmHg in 7 min of injection This was correlated with a concomitant decline in shivering to 55% of the preinjection level

Tyramine when administered i.v. ... mg/kg produced a marked increase in ... level from 0.36±0 05 to 0.51±0.14 μg/l ... not significant) Plasma adrenaline was ... ing 0 42±0 20 before and 0 45±0 18 μg/l ... the treatment

Table 2 shows the mean maximal ... T_b and T_f in the pigeon following ... trahypothalamic injections of noradrenaline ... tyramine in the cold environment The hypo... response to NA (5 μg/μl) was similar to that ... documented in the pigeon in similar ... (Hissa & Rautenberg 1974). No thermo... sponses to tyramine (10 μg/μl) was obtained ... injection in the same area The schematic ... tation of the injection area is shown in Fig ...

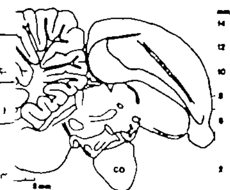
DISCUSSION

Since our present results with tyramine ... patible with those obtained by others with ... al injections of NA in birds (e.g. Allen & ... 1967 Freeman 1970 Hissa & Palokangas ... Hissa et al 1975 Pyörnilä et al 1976) both ... cold and warm environments they tend to ... that these effects are attributable to the ... release of NA Actually a slight elevation ... NA and A levels were demonstrated after ... injection Tyramine like NA abolished ... and elicited vasodilatation in the pigeon ...

Table 2 *Differences in the effects of intrahypothalamic injections of noradrenaline (NA 5 μg/μl) ... tyramine (Ty 10 μg/μl) in the same locus of the hypothalamus at 4°C*

11 measurements with 8 birds

	Mean max deviations in T (°C)	N	Time (min)	Mean max deviations n T (°C)	Time (min)	N
NA	−0.8±0 27	11	24±1 4	+0 2±0.55	16±2 1	11
Ty	−0 1±0 09	11	12±2 1	−0 1±0 19	1 ±0 8	11



Sagittal drawing through the pigeon brain ac
o the Atlas of Karten & Hodos (1967) indicating
here noradrenaline (5 μg/μl) and tyramine (10
ere unilaterally injected CO=chiasma opticum.

more the response to tyramine was at
l after inhibition of the endogenous syn-
f NA with MpT In a warm environment
ramine as with NA injections T was slight
ted along with an increase in heart rate (see
et al 1976). Likewise the observation that
tor blockade abolished almost completely
pothermic effect of tyramine is in accord with
ained in birds with NA and α-blockades
& Marley 1967 Hissa et al 1975)

total absence of thermoregulatory response
ed after intrahypothalamic administration of
ne could be due to the small dose injected
cated by the results obtained with intraven-
injections of tyramine in the cat (Metcalf &
1975). Probably tyramine after intra-
alamic injection did not reach a sufficient
of neurotransmitter compartments in the
vesicles to manifest the effects. Further
it is also possible that due to the immediate
ation deamination of released NA there were
equate number of neurotransmitters available
ulate the postsynaptic tes of the neuronal
ay

results are at least partly at variance with
of El-Halawani et al (1970) who found en-
d EMG activity after tyramine injection in the
n Paradoxically however both VO_2 and T
reported to be decreased. Apart from being
species differences, we find no other e pla-
for this discrepancy

le several schemes could be envisaged to ac
for the inhibition of shivering after tyramine
administration one that appears most plausible is
what is indicated by the recent findings of Marshall & Stoner (1979) with the rat and with the pigeon observed in our laboratory (Hohtola et al. 1980). A very close correlation between the decreased or increased blood pressure changes and abolishment of shivering was obtained. In the present work tyramine was found to produce an increase in blood pressure as well which might result in the changes in baroreceptor reflexes leading to the abolishment of shivering, as observed after i v NA injections in the pigeon (Hohtola et al 1980).

The neurogenic reflex vasodilatation elicited by substances that increase blood pressure is well documented (see Willems & Bogaert 1978) Such a mechanism is quite feasible and could explain the observed increase in foot temperature especially in the cold after administration of tyramine The vasodilatatory effect of tyramine has also been demonstrated in mammals (Burn & Tainter 1931 Dadkar et al 1977).

To summarize the present experiments tend to indicate that the effects obtained with tyramine both in warm and cold environment can be attributed to its effect caused indirectly via endogenous release of NA Furthermore our results support the original hypothesis of the role of NA in thermoregulation of birds. There was a fall in T in the cold environment, which t least partly is due to the decline in shivering and increased heat loss. In the warm environment a slight elevation in T might be a result of the excessively stimulated cardiac activity That NA was indeed involved is supported by the results obtained in experiments performed with the animals after reduction of their catecholamine pools. The direct central effect either on shivering motor centre or the blood pressure regulatory centre remains to be elucidated by attempt ng to block the effects of tyramine with injections of an α-blocking agent centrally

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…brane and biochemical alterations after denervation …uring reinnervation of mouse skeletal muscle

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SELLIN L C LIBELIUS R LUNDQUIST I TÅGERUD S & THESLEFF S Membrane and biochemical alterations after denervation and during reinnervation of mouse skeletal muscle Acta Physiol Scand 1980 110 181 186. Received 21 Jan. 1980 ISSN 0001 6772 Department of Pharmacology University of Lund, S eden.

Denervation of the extensor digitorum longus (EDL) muscle of the mouse by either nerv crush or nerv section produced reduction of the resting membrane potential (E_m) alterations in the properties of muscle fibre action potential and the development of tetrodotoxin (TTX)-resistant action potentials. These changes in membrane electrical properties were accompanied by an increase in the endocytic activity of the muscle and an increase in the activities of the lysosomal enzymes cathepsin D and N-acetylglucosaminidase (NAGA) Reinnervation of muscle was indicated at 9 days after nerv crush by the presence of miniature end-plate potentials. The recovery of membrane electrical properties, beginning with the onset of reinnervation, were not temporally related The E_m increased in tw stages, an early rapid repolarization and later slower repolarization. The muscle fibers were sensitive to the blocking action of TTX by 12 days after nerve crush whereas the rate of rise (dV/dt) of the action potential did not approach values of innervated muscles until 21 days Reinnervation resulted in decrease in endocytosis and decrease in the activities of cathepsin D and NAGA toward innervated values by 21 days after nerve crush The results suggest that membrane alterations after denervation and during reinnervation may occur by endo- and exocytosis of membrane constituents and that the lysosomal system may play role in the breakdown and/or recycling of these structures.

Key ords Denervation, reinnervation, membrane electrical properties endocytosis, lysosomal enzymes

…ation of mammalian skeletal muscle pro… alterations in the electrical properties of the …mma. Among these are a fall of the resting …ane potential (E_m), alterations in the muscle …action potential and the development of …toxin (TTX)-resistant action potentials …eff 196… Redfern & Thesleff 1971 …b). The …ization of the membrane and the develop… of TTX-resistant action potentials were …to depend on de novo protein synthesis after …ation, hile the alteration in the muscle fibre … potential appeared to be independent of new …ns (Grampp, Harris & Thesleff 1972).

…removal and insertion of membrane proteins …ccur by the process of endo-and exocytosis. …rate of endocytosis as measured by the up… of xtracellular marker molecules, was shown … denervation of skeletal muscle (Libelius et al. … In addition, denervation is reported to result in increased activities of lysosomal enzymes (Weinstock & Iodice 1969) which may be involved in the break-down and/or recycling of membrane constituents

The pu pose of the present study was to correlate the changes in membrane electrical properties after denervation and during reinnervation with concomitant changes in the rate of endocytosis and the activities of lysosomal enzymes Some of the results have been presented in preliminary communication (Sellin et al 1979)

METHODS

All experiments were conducted in vitro on the extensor digitorum longus (EDL) muscle of adult male NMRI mice Surgical procedures were done using diethyl ether for anesthesia. Chronic unilateral denervation was performed by removing 3-4 mm section of the deep peroneal nerve about 10 mm from its entrance into the muscle For rea…

nervation studies a 1 mm section of the nerve was crushed in the same position using a fine tipped forceps Control muscles were obtained from the contralateral un operated limbs At 3 6 9 1 14 and 21 days after nerve crush or section the muscles were examined for electrophysiological properties rate of uptake of macromolecular tracer or lysosomal enzyme activities

Electrophysiological properties At the various times after nerve crush or section the EDL muscles were excised under a continuous flow of oxygenated (95% O_2-5% CO_2) Krebs-Ringer solution having the following composition in mM NaCl 135 KCl 5 CaCl 4 MgCl 1 Na HPO 1 NaHCO 15 dextrose 11 The pH of the solution was 7.2–7.3 The muscles were pinned through their tendons stretched to approximately their resting lengths placed in a heated (30±1°C) chamber with a volume of 25 ml and suffused at a rate of 4 ml/min After a 30 min equilibration period muscle fibres were examined for resting membrane potentials and action potentials. In addition miniature end-plate potentials were recorded from some fibres to indicate the presence of reinnervation For these measurements glass microelectrodes filled with 3 M KCl and having tip resistances of 10–15×10⁶Ω were used Amplification and recording of potential were made using standard techniques (Katz & Thesleff 1957) The procedure for recording action potentials is described in detail elsewhere (Sellin & Thesleff 1979)

Uptake of H-inulin Animal were killed by cervical dislocation at various times following nerve crush or section The EDL muscles were excised and mounted on perspex holders kept in an oxygenated Krebs-Ringer solution (22°C) as described above but with mM CaCl Two muscles from each of the two experimental conditions and the four corresponding control muscles were mounted on the same holder After mounting the muscles were placed in an oxygenated incubation solution at +37°C containing 3 0 μCi/ml of H inulin (The Radiochemical Centre Ltd Amersham England) and incubated for 40 min During the incubation the solution was continuously bubbled with 95% O_2 5% CO_2 supplied through a peristaltic pump (flow rate ca 12 ml/min) to give standardized oxygenation and stirring conditions After incubation the muscles were washed in Ringer solution at +4°C for 4 h with the solution being changed each hour The muscles were then blotted on filter paper weighed and solubilized in 0 7 ml Soluene- 100 (Packard Instrument Company Inc Downers Grove Ill USA) over night at room temperature Radioactivity was measured by liquid scintillation counting using 10 ml of a toluene PPO-POPOP mixture Counting efficiency was calculated from an internal standard with H toluene (Packard Instrument Company Inc Downers Grove Ill USA)

Determination of lysosomal enzyme activities Cathepsin D activity was measured at pH 4 0 with haemoglobin as the substrate slightly modified from Barrett (1972) EDL muscles were homogenized in 3 ml ice-cold 0 3 mol/l sucrose in an Ultra Turrax homogenizer (Jank and Kunkel Staufen Germany) to yield a homogenate of approximately 0.5% muscle tissue (wet weight) The incubation mixture (0 4 ml) contained 0.2 ml muscle homogenate and 2% haemoglobin in a 0.25 mol/l sodium acetate buffer (pH 4 0) with 0 1% Triton X 100 The incubation was

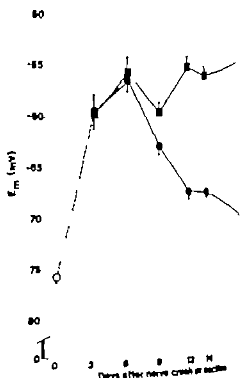


Fig 1 Resting membrane potential (E_m) (mean N= 4 706) in millivolts (mV) recorded from fibres in the extensor digitorum longus muscle mouse as a function of days after crush (filled circles) section (squares) of the deep peroneal nerve The circle indicates the E_m of normally innervated muscle

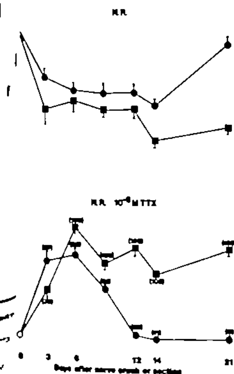
carried out for 120 min at +37°C and stopped by addition of 2 ml 3% TCA After centrifugation the clear supernatant was processed in the Folin Lowry reaction (Barrett 1972) Appropriate blanks and standards were included

N-acetyl-β-D-glucosaminidase activity was after the muscles had been homogenized as above Determination of N-acetyl-β-D-glucosaminidase with 0 1 mmol/l 4-methylumbelliferyl-2-acetamido-deoxy β-D-glucopyranoside as substrate was in a mixture of 0 075 ml muscle homogenate and 0 05 mol/l sodium citrate (pH 4.5) in the presence of Triton X 100 The reaction was carried out for at +37°C and terminated by the addition of 2.5 ml glycine buffer (pH 10 6) The fluorescence was in an Aminco Bowman spectrophotofluorometer excitation wavelength of 360 nm and an emission wavelength of 450 nm Appropriate blanks and were included

Protein determination were performed according Lowry et al (1951)

RESULTS

Electrophysiological properties Denervation of extensor digitorum longus (EDL) muscle of mouse produced significant alterations in the



Rate of rise (mean ± S.E. N 24–206) of the action potential (dV/dt) in volts per second (V/s) d from surface fibres in the extensor digitorum muscle of the mouse as function of days after rush (filled circles) or section (squares) of the deep l nerve. The figure is divided into tw parameters. of rise in normal Krebs Ringer solution (N.R.) and Krebs-Ringer solution containing 10^{-6} M tet- (N.R. 10^{-6} M TTX). The figures within paren- the percentage of fibres (N 31–103) respond- action potentials in the presence of TTX and the ± S.E. are based on these fibres only. The open indicate the rate of rise of normally innervated

ties of the sarcolemma. These changes were in magnitude and time-course regardless of er denervation was accomplished by nerve or section. Complete denervation was indi- at 3 and 6 days after surgery by the lack of e twitch after nerve stimulation and the ab- of miniature end-plate potentials (m.e.p.p.s) ind idual muscle fibres. The resti g mem- potential (E_m) was reduced from a control ated) value of -75.8 ± 0.4 mV to -56.6 ± 1.1 nd 55.9 ± 1.5 mV at 6 days after nerve crush ection, respecti ely (Fig. 1). Denervation also ced alterations in some properties of muscle

fibre action potentials (Fig.). For example 6 days following nerve crush or section the rate of rise (dV/dt) of the action potential decreased in both the nerve-crushed (596 ± 15 V/s) and the nerve-sec tioned ($574\pm$ 4 V/s) muscles from an initial inner vated alue of 758 ± 14 V/s. Similarly the threshold potential for action potential generation increased from -41.5 ± 0.9 mV to -47.2 ± 0.9 mV n dener vated and to -45.3 ± 0.8 mV in nerve-crushed muscles (9 day values).

At 3 days after denervation 70–90% of the muscle fibres examined had developed resistance to the blocking action of tetrodotoxin (TTX) and by 6 days all the fibres. The dV/dt of the TTX-resistant action potentials increased to peak values reaching 206 ± 13 V/ (nerve crush) and 273 ± 13 V/s (nerve section) at 6 days after denervation (Fig. 2).

Reinnervation was apparent in the nerve-crushed EDL muscles at 9 days after denervation with the appearance of m.e.p.p.s in individual muscle fibres along with weak muscle twitches after nerve stimulation. M.e.p.p.s and muscle twitches were not observed in any of the nerve-sectioned muscles during the entire time-course of the study. With the onset of reinnervation, there was a slight increase in the E_m for the nerve-crushed muscle ($-63._\pm0.7$ mV) compared to the nerve-sectioned muscle (-59.8 ± 0.9 mV). By the 1?th day after nerve crush the E_m of the reinnervating EDL had increased substantially to -67.7 ± 0.8 mV while the nerve-sec tioned muscle remained at -55.3 ± 0.9 mV. After this time the E_m of the reinnervating EDL increased at a slow rate and was near that for normally inner vated muscle at 21 days after nerve crush (Fig. 1).

The recovery of the muscle action potential during reinnervation can be divided into two events the recovery of action potential properties *per* (e.g. dV/dt) and the recovery of muscle fibre sensitivity to the blocking action of TTX. Between 9 and 12 days after nerve crush the reinnervating EDL became rapidly sensitive to TTX. This is indicated by the reduction in the number of fibres with TTX resistant action potentials from 87% at 9 days to 26% at day 12 and by a reduction in the dV/dt of the fibres with TTX-resistant spikes (Fig. 2). The dV/dt of the action potential in TTX-containing solution decreased from 206 ± 3 V/s at 6 days after nerve crush to 16 ± 3 V/s at 12 days. In contrast to the TTX-sensitivity the dV/dt of the action potential in normal Krebs-Ringer solution stayed at values similar to those in denervated fibres at 14 days

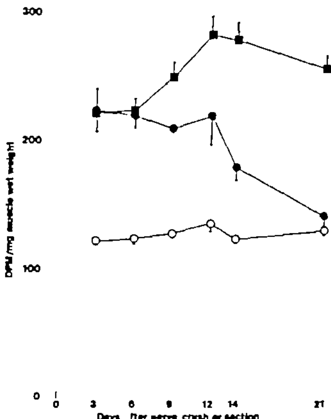


Fig 3 The uptake (mean ± S E.) of H Inulin in disintegrations per minute per milligram muscle wet weight (DPM/mg muscle wet weight) by the extensor digitorum longus muscle of the mouse as a function of days after crush (filled circles N=8) or section (squares, N=8) of the deep peroneal nerve The open circles indicate the uptake of normally innervated muscle (N=16)

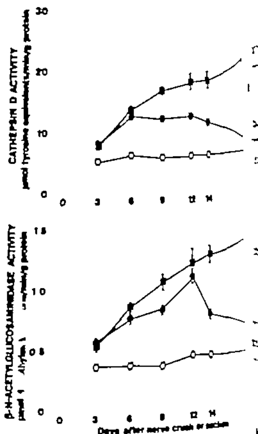


Fig 4 The activities (mean ± S E) of the lysosomal enzymes cathepsin D (upper panel) expressed as tyrosine equivalents liberated/min/g protein and β-N-acetylglucosaminidase (lower panel) expressed as 4-methylumbelliferone liberated/min/g protein in innervated control muscles (open circles, N… nervating nerve-crushed muscles (filled circles N… chronically denervated muscles (squares, N=… as a function of days after nerve crush or section

after nerve crush (56?±17 V/s) and did not approach values of innervated muscles (758±14 V/s) until 21 days after nerve crush (705±18V/s) (Fig ?) The recoveries towards values of innervated muscles of threshold potential and overshoot of the action potential during reinnervation occurred between 14–21 days

Uptake of H Inulin The endocytic activity of EDL muscles after denervation and during reinnervation as measured by the uptake of H-inulin is shown in Fig. 3 Denervation by both nerve crush or section produced almost a 100% increase in the uptake per mg wet muscle weight at 3 days after denervation The nerve sectioned muscles showed a further increase in uptake between days 6 and 12 followed by a plateau at about 150% of control values by 21 days In contrast the nerve-crushed muscles showed no increase between days 6 and 12 and then slowly decreased their uptake after 1…

days reaching values similar to normally … muscles by 21 days

Lysosomal enzyme activities Denervation by either nerve crush or section produced … creases in the activities of the muscle ly… enzymes N-acetylglucosaminidase (NAGA) … cathepsin D (Fig 4) The activities of … enzymes in the nerve sectioned muscles … steadily reaching apparent plateau values at 21 days However the muscles whose nerve … been crushed showed a slower or no increase … activities between 6 and 12 days and decr… thereafter By 21 days after nerve crush, the activities of NAGA and cathepsin D were at … innervated values while the nerve sectioned muscles showed 3–4 times higher activities.

SION

that some denervation-induced alterations rane electrical properties are dependent on ynthesized proteins (Grampp et al 1972) that these proteins are incorporated into cle membrane It is likely that this type of ne turnover may be explained by the pro- endo- and exocytosis The increase in the of ³H-inulin in denervated skeletal muscle n the present study was similar to that re previously for uptake of extracellular mar muscle after denervation by the vesiculation rnalization of surface membrane primarily tubular region (Libelius et al 1978 Libelius on & Lundquist 1979). Since in the present ere was no change in muscle weight relative vated muscle at 3 days after nerve section reased uptake per mg muscle clearly indi- increase in endocytosis. Within that period the alterations in membrane electrical prop- ypical of denervation occurred The further e in uptake per mg muscle between 6 and 21 ter nerve section, was complicated by a con- nt loss in muscle weight, which was pre to have been decrease in fibre olume than number (Pellegrino & Franzini 1963). s, when the data were plotted as total DPM muscle (not shown) against days after denerva- he uptake of the nerve-section muscles re- d at a high, but constant, level between 3 and ys and thereafter displayed an apparent de at a slow rate Therefore denervation in- l high rates of endocytosis which was main- until at least 12 days after nerve section. No nitial changes in the membrane electrical rties were observed through 21 days after section beyond the initial denervation-in- l alterations, which occurred within the first ays.

the nerve-crushed muscles, the uptake of ulin per mg muscle weight after denervation ased in a fashion similar to the nerve-section les. While the uptake per mg muscle increased ween 6 and 12 days in the nerve-sectioned mus- the uptake per mg muscle in the nerve-crushed les remained constant during this same period. the muscles in both preparations decreased in ht during this time It may be concluded that the les which were reinnervated decreased their ocytic activity This decrease occurred between 6 and 9 days after nerve crush At times later than 12 days after nerve crush the decrease in uptake per mg muscle resulted from the combined effects of a decrease in total uptake per muscle and an in- crease in muscle weight. It is thus apparent that the increased endocytic activity initiated by denerva- tion is reversed by reinnervation.

Reinnervation occurs between 6 and 9 days after nerve crush because miniature endplate potentials were present at 9 days The results demonstrated that recovery of some membrane electrical proper ties d ring reinnervation were not temporally re lated. That is, the time-course of recovery differed between individual electrical properties e g. E_m rate of rise (dV/dt) of the muscle fibre action poten- tial and TTX sensitivity The repolarization of the membrane during reinnervation was similar to that previously reported (McArdle & Albuquerque 1973) The present result suggests that the increase of the E_m occurs in two stages a rapid increase at the onset of reinnervation between 6 and 1 days, and a slower increase between 12 and 21 days. In addition dV/dt and TTX-sensitivity did not recover at the same rate as shown previously in a prelimi- nary report (Sellin & McArdle 1977) By 12 days after nerve crush the reinnervated muscles like in- nervated muscle were almost completely sensitive to the blocking action of TTX. In contrast the dV/dt in the absence of TTX remained at a value similar to chronically denervated muscle through 14 days after nerve crush and only approached innervated values at 21 days

The difference in recovery rates between and within the above parameters may be interpreted in the following manner The changes in membrane electrical properties occurring during reinnervation depend on the morphology and lipid composition of the membrane in addition to the removal and inser tion of specific membrane proteins. It appears likely that the early rapid repolarization of the membrane and the early abolishment of TTX-insensitivity are related to the removal of proteinaceous material responsible for sodium permeability in the muscle membrane. In regard to E_m it is believed that the depolarization caused by denervation is, at least in part, secondary to an increase in the sodium leak permeability of the muscle membrane (Robbins 1977). TTX-insensitive channels are presumably voltage sensitive sodium channels The late slower repolarization could result from conductance changes which similar to dV/dt may depend on the

relationship of ion channels to their environment It is interesting to note that the decrease in membrane resistance which reflects an increase in ionic conductances occurs at the later stages of reinnervation after an early increase in E_m (McArdle & Albuquerque 1973)

Earlier studies suggested that increased endocytosis in denervated skeletal muscle may activate lysosomal mechanisms thereby fostering degradative processes (Libelius et al 1978) The present study provides further evidence for at least a temporal relationship between endocytosis and the activity of the lysosomal system The activities of the proteolytic acid hydrolase cathepsin D and the acid glycosidase N acetylglucosaminidase (NAGA) increased steadily after nerve section while levelling off with the onset of reinnervation and then decreased to near innervated values by 21 days after nerve crush in conformity with changes in endocytic activity and membrane electrical properties The exact mechanisms by which the lysosomal system is implicated in degeneration and recovery of the muscle in denervation reinnervation remain to be elucidated It seems likely however that the activation of the lysosomal system by endocytosis is responsible for the breakdown and/or recycling of membrane constituents

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...hological and histochemical properties ...ngue muscles in cat

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HELLSTRAND E. Morphological and histochemical properties of tongue muscles in cat. Acta Physiol Scand 1980. 110: 187-198. Received 4 Jan. 1980. ISSN 0001-6772. Department of Physiology, Karolinska Institutet, Stockholm, S... eden.

The morphology of the tongue muscles was studied by in situ dissection as well as by histological and histochemical methods. By means of the latter an anatomical reassessment of attachment and fiber courses was made. The histochemistry was studied in sections stained for myofibrillar adenosine triphosphatase (mATPase), succinic dehydrogenase, NADH diaphorase, phosphorylase, esterase, glycogen and lipids. Fibers of type I and type II were identified, and the latter were subdivided into II (highly glycolytic), II (intermediately glycolytic and lipolytic) and II_{m} (highly lipolytic). I... the *extrinsic* muscles, the fibers were 19-25% type I (mean diameter 77 μm) and 75-81% type II (37 μm); the three type II subgroups appeared in equal proportions, each accounting for 22-30% of the total fiber amount. Pars longitudinalis superior m. hyoglossi and pars longitudinalis inferior m. styloglossi contained only type II fibers, mainly type II... (67% and 46% respectively), of diameters like those in m. hyoglossus and m. styloglossus. The *intrinsic* muscles also consisted entirely of type II fibers (23 μm). II_{m} fibers predominated in m. verticalis (83%), which has only 10% II... and 6% II..., whereas the fiber composition of m. transversus was more balanced: 37% type II..., 32% II... and 31% II_{m}.

Key words: Tongue muscles, cats, anatomy, histochemistry, fiber classification

...ongue of the cat, with mainly intraoral action ... mastication (the type I category according to ... & Baggett 1971), is provided with muscles ...d *ext...sic* or *intrinsic* according to whether ...un partly or totally within the tongue. Func...ly the extrinsic muscles are usually classified ...truders or retractors and the intrinsic as shap... modelling agents.

...rphologically the muscles of the tongue have ... studied extensively (e.g. Ludwig Ferdinand ..., Edgeworth 1935, Abd-El-Malek 1938, Dabe... 1951, Reighard & Jennings 1961, Doran 1975). ...he resulting reports often differed about the ... of insertion and fiber courses of individual ...cles. Precisely which muscles contribute to ...h movement of the tongue has therefore been ...tar.

...w, however, the histochemical techniques ...monly used to examine enzyme activities and ...trate contents within muscle fibers can be ...bined with conventional histology and in... itu ...ections, so that the course of fibers from indiv...idual muscles can be determined more reliably than before. The present study of morphological and histochemical properties of the tongue muscles employed such an approach. The results are intended to serve as basis for further studies on the functional properties of these muscles.

MATERIAL AND METHODS

Basic information on tongue muscle morphology was obtained from textbooks (e.g. Reighard & Jennings 1961) and evaluated through in situ dissections of 8 cats under ... Zeiss dissection microscope. Some of the material dissected had been fixed in 10% formol. The general view of the muscle topography thus obtained guided the subsequent histological and histochemical studies.

Histological procedure. Tongues from 5 adult cats (2.3-4.6 kg) were dissected along the septum medianum, fixed in 10% formol and embedded in paraffin. Frontal, sagittal and transverse sections were cut and stained with (1) iron-hematoxylin and eosin and (2) Weigert-van Gieson solution. For further details on the staining procedures see Romeis (1968).

Histochemical procedure. Biopsies were done on the tongue muscles of 12 adult cats (2.5-4.5 kg) and the

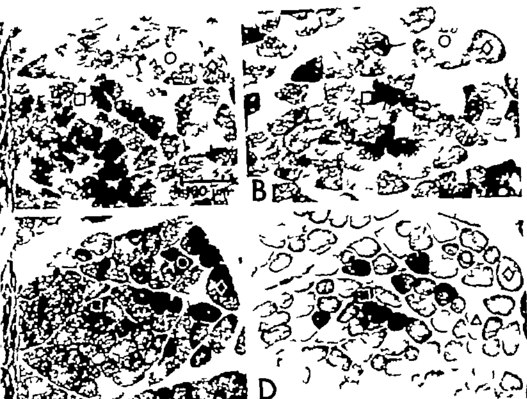


Fig. 1. Histochemical stainings of m. styloglossus secutive cross sections stained for (A) myofibrillar ATPase pH 9.4 (B) myofibrillar ATPase pH 4... (C) myofibrillar ATPase pH 4... (D) NADH diaphorase ... succinic dehydrogenase. Fiber type marked as Δ = II ◇ = II$_B$ □ = II$_A$ see text

pecimens were immediately frozen in isopentane previously cooled in liquid nitrogen. Sections 1 –16 μm thick were cut on a microtome at −20°C and stained.

For the parts of the extrinsic muscles running outside the tongue mass biopsy material was taken from the origin of the muscles up to the middle part of the bellies (i e where the muscles pass into the tongue). For biopsy of the parts of the extrinsic muscles running within the tongue and for the intrinsic muscle cubes (side 15 mm) were cut from the anterior middle and posterior parts of the tongue. All samples were sectioned on the microtome in three perpendicular planes so as to expose the muscles in cross section (e g in the frontal plane para longitudinalis superior m. hyoglossus and pars longitudinalis inferior m. styloglossi in the sagittal plane m. transversus and in the transversal plane m. verticalis).

Stainings. (1) myofibrillar ATPase also called activated myosin ATPase (Padykula & Herman ...) after pre-incubation in sodium barbital buffer at pH ... in barbital acetate buffer at pH 4.6 and 4.2 (Dubowitz & Brooke 1973) ( ) succinic dehydrogenase (Nachlas ... 1957) (3) NADH diaphorase (Scarpelli, Hess & ... 1958) (4) phosphorylase a (Takeuchi & Kuriaki ... Chayen Bitensky & Butcher 1973) (5) glycogen (... & Millican 195) (6) esterase (Holt & Withers 19... lipid (Carleton & Drury 1957 ... ght modifications



Histochemical stainings of an styloglossus. Consecutive cross sections stained for (A) phosphorylase (B) (C) lipids, (D) esterase. Fiber types marked as in Fig. 1

...cording to Nystrom (1968), for further details see (1968).

...ation and density measurements. The stained tissues ...ere examined as slides under the micro... ed in micrographs.

...he histochemically stained fibers the relative den... ...ere measured from panchromatic black and ...hite ...atives (Panatomic X, Kodak) by means of an ... recording microdensitometer (Model MK III C, Loebl & C... Ltd), ...hich operates on ... double ...ight system connected to ... photomultiplier and ... density directly and linearly. Baseline ...as set ...e unexposed part of each negative.

...ing reactions. As ... representative ...xample of the ...e activity and substrate content of the muscles ...A, the histochemical staining properties of an stylo... ...re shown ...n Fig. 1 A–E and Fig. 2 A–D (all are ...cutive cross sections). The following presentation of ...staining reactions shows ... ...thin brackets the mean ...e density or ranges of density of the fibers ...tained.

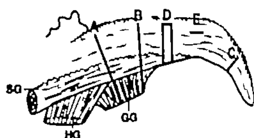
...fibrillar ATP...se. After preincubation and staining ...9.4 ...s ... fiber types could be distinguished: type I ...ow enzyme activity (0.68), and type II ...with high ...ty (1.45–1.38). Preincubation at pH 4.2 showed the ... type I high (1.28–1.00) and type II low (0.11). ...preincubation at pH 4.6, three levels of activity were seen: high (1.33), intermediate (0.95) and low (0.78). Type I fibers showed only high enzyme activity whereas type II fibers exhibited all these levels. See Fig. 1 A, B and C for the staining reactions at pH 9.4, 4.6 and 4.2 respectively.

Succinic dehydrogenase and NADH diaphorase. Fig. 1 E shows the staining reactions for the succinic dehydrogenase. The distribution of the formazan granules within the fibers varied, disclosing 3 categories of fibers: those with ... largely diffuse distribution of granules, those with diffuse granules plus ... clear subsarcolemmal formazan activity and those showing interior ...treak... of formazan together with the subsarcolemmal and diffuse granules. Every type I fiber belonged to the third group while type II fibers were found in all three.

The staining pattern of NADH diaphorase (Fig. 1 D) was essentially the same, although the granules were denser and the interior streaks more pronounced than with the dehydrogenase staining. This might reflect the different distributions of these two enzymes within the muscle cells, since succinic dehydrogenase occurs only mitochondrially whereas NADH diaphorase is found microsomally as well (de Duve, Wattiaux & Baudhuin 196...). The type I and II fibers were grouped as for the dehydrogenase.

Phosphorylase ... and glycogen. The activity of phosphorylase ... (active phosphorylase, see Chayen, Bitensky

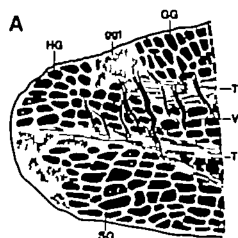


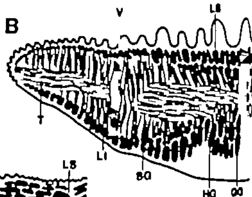


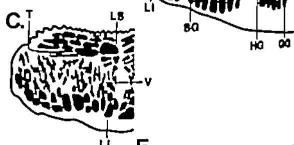
A. B C. FRONTAL SECTION THROUGH THE RIGHT HALF OF THE TONGUE

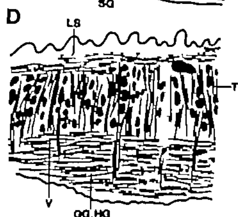
D SAGITTAL SECTION

E. HORIZONTAL SECTION









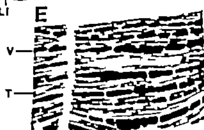


Fig 3 The cat's tongue Dorsal and lateral views of the tongue illustrated together with frontal (A B C) sagittal horizontal (E) sections through the right half of the tongue *GC* m genioglossu *HC* m hyoglossus styloglossu *LS* pars longitudinalis superior m hyoglossi; *LI* pars longitudinali inferior m styloglos transversus *V* m verticali *gxl* glandulae linguales

& Butcher 1973) In the muscle fibers is indicated by Fig 2 A Type I fibers had low acti ity (0 70) and type II intermediate (1 08–0 93) to high (1 18)

The taining for glycogen content (Fig B) col I fibers les darkly (0 60) than type II fibers (1 1 reaction like that for ph ylase activity Rare

-t were high in phosphorylase proved low in This glycogen depletion more likely arose from hemical procedure than from exhausting work, work also depletes the amount of active phos- (Cori 1945 Kugelberg & Edström 1968).

and lipids The type I fibers showed only high activity (1 08); type II had high (1.08), inter 0 95) and low (0 48) levels (Fig 2 D).

terase levels were related to the lipid content s, fibers high in esterase activity storing more those ith low enzyme activity Type I fibers ndly for lipids (1 43) type II ere highly (1 34), ately (1 32) or lightly (0 85) stained

The fiber sizes in the tongue muscles were from micrographs of cross sections stained for (pH 9 4) Lesser fiber diameter (Dubowitz & 973) as the index of fiber size (see also Brooke 1969)

cal method Different muscles or fiber groups mpared with Student -test The differences red insignificant (*P*<0 01) see also Bailey 1959

TS

logy

ngue muscle morphology was studied by dissection and by histological and histo- l analyses of biopsy materials Data on each are presented below together with remarks er investigations To begin with *general* w Fig. 3 offers dorsal and lateral views of tongue s well as drawings of frontal sagit horizontal sections through the right half of gue.

dorsal iew of the tongue in Fig 3 shows the surface with the sites of the frontal (A B C) (D) and horizontal (E) sections indicated papillae plica glossoepiglottica mediana ull central fold ising from the dorsal tongue to the epiglottis) and the cranial surface of iglottis are sketched.

lateral iew of the tongue includes sites of s (A–E), and topography of the extrinsic s, with the ventrolateral part of the tongue from its mucous membrane to larify the rela- among the individual muscles.

frontal section through the right half of the e demonstrate the posterior (A) middle (B) terior (C) parts. The drawings were based on graphs of histochemically stained cross sec and retain the true relative proportions. In A, oglossus occupies the ventral part, m genio- s the dorsomedial and medial parts and m. osus the area between these two muscles, its fascicles being distributed obliquely to the vertical plane.

In B the bundles of m. genioglossus and m. hyoglossus, comprising both type I and type II fibers (see Histochemistry below) run medial to the mixed fiber portion of m styloglossus. Lateral to it pars longitudinalis inferior m. styloglossi (type II fibers only) forms a thin layer that narrows further as it nears the side of the tongue I order to exemplify origins and insertion areas of m transversus and m verticalis in the same picture both muscles are shown together although in iew of their topography (see below) only one muscle should be seen

In C the fibers operating the tongue tip (all type II) are shown Although small fiber bundles of the transverse and ertical muscles are present fibers of the longitudinal muscles predominate

The sagittal ection (D) shows the muscle topography half way along the tongue near the midline The drawing was made from sections stained histologically a procedure that usually deforms muscle fibers more than the histochemical treatment.

The horizontal section (E) of the medial part of the tongue s right half was cut just below the superior longitudinal fibers. It reveals the topography of m. transversus and m verticalis (specimens histochemically stained)

Protruding the tongue

M genioglossus Its fibers were found to originate from the tubercle close to the symphysis of the lower jaw dorsomedial to the origin of m ge niohyoideus. The muscle passes within the lingual frenulum into the ventral part of the medial aspect of the tongue The fibers then fan out in the vertical plane forming a thin muscle sheet which is inserted into the submucosal layer immediately be low the superior longitudinal fibers of m. hyoglossus (see below) The insertion area extends from the anterior to the posterior part of the tongue Posteriorly the muscle forms the medial part of the tongue root, together with m. hyoglossus. Anteriorly the fibers run more vertically to reach their insertion points terminating at least 1.5–2 cm from the tip s edge The anterior portion of the muscle relates lateral to the fascicles of m styloglossus.

According to e g Abd-El-Malek (1938) and Dabelow (1951) the genioglossal muscle also inserts into the hyoid bone the epiglottis and the tongue tip No fibers could be traced to these structures in

this study however The present findings concur with those of Doran & Baggett (197?) who denied that the anterior reach of the muscle extends to the tip

The two genioglossal muscles of the tongue were strictly separate None of the fibers from one inter mingled dorsally with those from the opposite side (cf Dabelow 1951 Reighard & Jennings 1961)

Retracting the tongue

1 *M hyogl ssus* The muscle arose from the body of the hyoid bone and from the proximal part of its cranial cornu (i e the lesser horn of human anatomy according to Reigh ird & Jennings 1961) It runs parallel to the midline of the neck making up the middle part of the tongue root with m genioglossus lying medial and m styloglossus lateral to it Most of the fibers spread obliquely funlike in the vertical plane parallel to m genioglossus They insert into the dorsal submucosa from its posterior to its middle portion Some fibers in contrast unite and run parallel to the dorsal tongue surface to in ert into the tongue tip forming its dorsal muscle layer and acting as the tip dorsoflexor Those fibers—previou ly termed m longitudinalis superior —appear as a thin transverse sheet under the dorsal tongue epithelium In thi study the muscle sheet is named pars longitudinalis superior m hyoglossi (see Histochemistry below) The hyoglossal muscle is paired

The origin of m hyoglossus has been variously attributed to the caudal cornu of the hyoid bone (greater horn Braus 1924 Cunningham 1964 Gray 1973) its cranial cornu (Abd El-Malek 1938) and its body (Reighard & Jennings 1961) This study found no fibers originating from the caudal cornu The inserting fibers have been said to intermingle with fibers of m longitudinalis inferior and m styloglossus (e g Cunningham 1964) but both of the hyoglossal muscle portions identified here were well separated from the other tongue muscles (see also Dabelow 1951)

? *M styloglossus* The muscle originates from the cranial cornu of the hyoid bone from the area around the tympanic bulla and from the styloid process Its fibers enter the lateral part of the posterior tongue forming the lateral part of its root with m hyoglossus lying both dorsal and medial to it and m genioglossus medially It runs parallel to the ventral epithelium in a tringular shape that shrinks anteriorly The main fiber portion lies in the middle of the forward half of the tongue A smaller enters the tongue tip to form its ventral mu... and act as the tip ventroflexor This f... here called pars longitudinalis inferior m ...glossi (see Histochemistry)

M styloglossus has a rather extensive origin compared to the genioglossal and hy... muscles Its two fiber portions were ... fine having no fibers mixed with m. hy... (cf Cunningham 1964) or m. genioglo... below 1951) Abd-El-Malek's (1938) rep... (Dabelow 1951) Abd El-Malek's (1938) the muscle winds around the lateral and ... parts of the inferior longitudinal muscle before the middle of the tongue postero-ante... was not borne out On the contrary the fiber... styloglossal muscle run in close formation ... only the medial one third of the tongue ... other two extrinsic muscles According to D... (1951) after entering the tongue root a medi... of m styloglossus runs to the opposite side ... tongue on its way mixing with fibers of m ... versus to end up attached to the styloid pro... the opposite side it thus constitutes a m... connection between the two styloid process... also Hesse 1875) This was not confirmed ... present study

Shapi g the tongue

1 *M transversus* The muscle consists of ... versely running fibers originating from the ... septum of the tongue and from the fibrous ... surrounding m genioglossus and m hyo... The fibers form thin sheets paralleling one ... in the frontal plane and inserting into the ... integument of the tongue The muscle fi... central part of the tongue but becomes shor... narrower toward the root and tip end D... some of the fibers are inserted between t... fascicles of pars longitudinalis superior m ... glossi Ventrally it runs above m stylo... with some fibers inserted among the late... cicles of pars longitudinalis inferior m. stylo...

As no fibers of the left or the right tra... muscles are seen to cros the midline of the ... not even where the median septum is ... the muscle is considered paired (cf Braus ... Dabelow 1951) Its clo e connection to the m... septum is well establi hed (e g Hesse 1875 ... wig Ferdinand 1884 ... & Markowski

951). No sign of the reported connection transverse fibers and the origin of m. us (Dabelow 1951) was found.

allel arrangement of the transverse sheets is easily demonstrated by sagittal and l sectioning. A plaiting of transverse and fibers as described by previous inves- was not seen except at the far edge of the mong the inserting fibers.

erticalis The muscle originates from al submucosa under pars longitudinalis m. hyoglossi, minor portions also come sheaths around fascicles of this muscle. It eets which are organized in parallel in the tane. The vertically running fibers are in- to the ventral submucosa or among the of the muscles in the ventral muscle layer ed.

pography of the muscle is clearly evident ntal and sagittal sections. Its extension is that of m. transversus their sheets of ernating.

longitudinalis superior and m. lon- inferior Previous studies considered uscles intrinsic with well-defined ori- er courses and insertions. M. longitudina- rior was said to originate from the os m, membrana hyoglossi and the dorsal membrane, its fibers forming the dorsal layer and being inserted into the tongue tip. tudinalis inferior was said to extend from hyoideum and epiglottis to the tongue tip posteriorly between m. genioglossus (me and m. hyoglossus (laterally), then anteriorly m. genioglossus and m. styloglossus. lose ypoglossal nerve and the lingual vessels present study defines both muscles different- superior longitudinal muscle was shown to ber portion of the hyoglossal muscle. The longitudinal muscle was found to extend e tongue tip to the origin of the styloglossal running in the lateral part of the tongue, to m. hyoglossus. Hence Dabelow's (1951) that short, longitudinal superficially run- bers belong to proper intrinsic muscle, ot only the longer more deeply located fibers le from extrinsic muscles was disproved as existence of m. longitudinalis proprius s. (Ludwig Ferdinand 1884. Nusbaum & Mar 1896) borne out.

er amount M. genioglossus consists of about 30...

1600 fibers, m. hyoglossus and m. styloglossus contain around 2000 each. The number of fibers forming a single muscle sheet (around 150 μm thick) of m. transversus or m. verticalis varies widely: the maximum (at the central part of the tongue) is around 1300–1400. Pars longitudinalis superior m. hyoglossi consists of about 700 fibers in the anterior part of the tongue where pars longitudinalis inferior has somewhat fewer around 600: only about one fourth of these anterior fibers reaches the dorsal and ventral tip.

Histochemistry

Muscle fibers that had similar histochemical staining reactions were grouped, then classified as type I or type II on the basis of their staining reactions for myofibrillar ATPase pH 9.4 (terminology of Fenichel & Engel 1963). An index number (1-3) added to the type symbol designated each group's staining reaction to succinic dehydrogenase: 1 diffuse spread of formazan granules, 2 distinct subsarcolemmal activity of formazan, 3 distinct interior streaks of formazan.

Table 1 summarizes the histochemical profiles of the groups. The I_{m} fiber appears to possess a high oxidative capacity together with high lipolytic and low glycolytic capacities. The reverse applies to the II. The II_{n} fiber is intermediate in all three respects and the II_{m} group is uniformly high.

One fiber from each group is labelled in Fig. 1 and to illustrate their histochemical profiles.

Fiber composition and size in extrinsic muscles

All 4 fiber groups are represented in these muscles with the type II varieties (II, II_{n}, II_{m}) clearly predominant. The mean diameter for type II fibers is around 36 μm and the mean for type I about 28 μm. The fibers designated II and II_{n} have similar diameters and are significantly larger (by about 10 μm) than the II_{m} group. The frequencies and sizes of type I and type II fibers are listed in Table 2.

M. genioglossus (Data collected from the part running outside the tongue mass.) No fiber group preponderates, each having about 25% frequency of occurrence. Cross section of the muscle showed no clustering of fibers of any group, only irregular scattering.

The mean diameter of the II_{m} group is small, around 27 μm, and its fibers are among the finest in the extrinsic muscles. The mean diameters of the II

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Retracting the tongue

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2 *M styloglossus* The muscle originates from the cranial cornu of the hyoid bone from the area around the tympanic bulla and from the styloid process Its fibers enter the lateral part of the posterior tongue forming the lateral part of its root with m hyoglossus lying both dorsal and medial to it and m genioglossus medially It runs parallel to the ventral epithelium in a tringular shape that shrinks anteriorly The main fiber portion lies in the middle of the forward half of the tongue A smaller ... enters the tongue tip to form its ventral ... and act as the tip ventroflexor This fiber ... here called pars longitudinalis inferior m ... glossi (see Histochemistry)

M styloglossus has a rather extensive origin compared to the genioglossal and h... muscles Its two fiber portions were con... fine having no fibers mixed with m hy... (cf Cunningham 1964) or m. genioglossus ... below 1951) Abd El-Malek s (1938) ... (Dabelow 1951) Abd El-Malek s (1938) ... the muscle winds around the lateral and ... parts of the inferior longitudinal muscle ... before the middle of the tongue postero-... was not borne out On the contrary the fibers ... styloglossal muscle run in close formation ... only the medial one-third of the tongue ... other two extrinsic muscles According to D... (1951) after entering the tongue root a ... of m styloglossus runs to the opposite side ... tongue on its way mixing with fibers of m ... versus to end up attached to the styloid pro... the opposite side It thus constitutes a ... connection between the two styloid process... also Hesse 1875) This was not confirmed ... present study

Shaping the tongue

1 *M transversus* The muscle consists of ... versely running fibers originating from the septum of the tongue and from the fibrous ... surrounding m genioglossus and m hyo... The fibers form thin sheets paralleling one ... in the frontal plane and inserting into the ... integument of the tongue The muscle fi... central part of the tongue but becomes ... narrower toward the root and tip each ... some of the fibers are inserted between the ... fascicles of pars longitudinalis superior m ... glossi Ventrally it run above m stylo... with some fibers inserted among the ... cicles of pars longitudinalis inferior m stylo...

As no fibers of the left or the right ... muscles are seen to cross the midline of the ... not even where the median septum is ... the muscle is considered paired (cf Braus ... Dabelow 1951) Its close connection to the ... septum is well established (e g Hesse 187... wig Ferdinand 1884 Nusbaum & Markowski

es or fascicles of very fine fibers such as cate a place of attachment for the lon- ibers. Rather the dorsal fibers spread : hyoglossal fibers in the root ending e body of the hyoid bone These fibers ate in the same plane as the fibers of m. s. During their course from the origin ually form distinct fascicles, easily iden- he middle of the dorsal tongue running e up to the tip The composition is mainly (67%), the rest being equal parts II and three fiber types are irregularly scattered e muscle portion

ht be expected the fiber diameters of this se hyoglossal muscle do not differ signifi- m those measured in the undivided muscle he tongue.

loglossus (Data collected from the part he tongue mass) The composition is 28% (31 μm) and 27% II (41 μm), followed by fibers at 22% (39 μm) and the I_{m} fibers at) μm). Distributions of all types are irregu-

longitudinalis inferior m styloglossus Sec of the tongue as described for pars lon- lis superior m. hyogloss revealed another portion made up exclusively of type II fibers d be followed from the ventral part of the tip h the lateral parts of the ventral tongue to the t the root its fibers ceased to be separate e medially lying styloglossus which consists type I and type II fibers but spread among fibers. Thus, both fiber portions are inserted e same points of origin with their fibers ced

fiber composition of this ventrolateral mus- *ntion* is primarily II (46%) with 40% II_{m} ly 15% type II All are irregularly scattered. diameters are about the same values found in yloglossal part outside the tongue

composition and ace ... muscles

fiber composition of these muscles is almost sively type II very rarely a single type I fiber d be found among the fiber bundles. The mean eters are about 23 μm, making these the finest of the tongue As with the extrinsic muscles, fibers are the largest and the II_{m} the small- Y II

ber group is the II at 37% (mean diameter 30 μm) followed by the II_{n} at 32% (76μm) and the II_{m} at 31% (20 μm). Although all three kinds of fibers are mixed together in fasci- cles, the fascicles inserting into the most lateral part of the tongue seem to have more II fibers than the other fascicles.

M verticalis The II_{m} fiber group is conspicu- ous representing 83% of the total composition (mean diameter 20 μm) The remainder consists of 10% II (28 μm) and 6% II_{n} (74 μm) The fascicles appear fairly homogeneous in cross section, the clusters of II_{m} fibers shot with fibers of the scarcer groups.

DISCUSSION

The tongue muscles attachments and fiber courses as reported here differ in part from earlier findings It is conceivable that the sampling techniques employed by previous investigators obscured cer tain aspects of the tongue muscle anatomy or that species differences among the animals studied were not taken into account. In any event, previous his- tological methods of tissue preparation and staining certainly deformed the tissues more than modern histochemical procedures. Compare for example Fig 3 D and E. the intrinsic muscles drawn from histological sections (D) are much less distinct than those drawn from histochemical sections (E). Only the histochemical treatment reveals unequivocally the parallel alternation of m transversus and m verticalis in sheets

The morphological findings presented here are compatible with the various functions of the mus- cles, such as tongue protrusion or retraction, tip flexion or changing the tongue s shape. If m. genio- glossus were attached to the hyoid bone—as stated by e g Abd El-Malek 1938 but here disproved— this muscle would have to subserve jaw opening (cf anatomy and function of m. geniohyoideus accord- ing to Reighard & Jennings 1961) No such function has ever been ascribed to it (Edgeworth 1935 Lowe 1976) If the same muscle were attached to the tongue tip (e.g. Dabelow 1951) it would have to be a tip ventroflexor a role rejected by Doran & Baggett (1972) in their reassessment of the anatomy of m. genioglossus. The present study found no such attachment, thus upholding their analysis and extending it to cat

The longitudinally running muscle fibers are here defined as belonging to the retract sys-

Table 1 *Histochemical fiber profiles of the tongue muscles*

Staining reactions with mean relative density or ranges of density shown within brackets

I_m	II	II_i	II_m	Enzyme or sub
Low (0.88)	High (1 45–1.28)	High (1 45–1 _8)	High (1 45–1.28)	Myofibrillar (0) pH 9 4
High (1.28–1 00)	Low (0 11)	Low (0 11)	Low (0 11)	Myofibrillar (0) pH 4 2
High (1.3)	High (1.33)	Intermediate (0 95)	Low (0 78)	Myofibrillar (0) pH 4 6
Diffuse sub-sarcolemmal in terior streaks	Diffuse	Diffuse sub-sarcolemmal	Diffuse sub-sarcolemmal Interior streaks	Succinic dehydrogenase (granule distribution)
Diffuse sub-sarcolemmal Interior streaks	Diffuse	Diffuse sub-sarcolemmal	Diffuse sub-sarcolemmal Interior streaks	NADH diaphorase (granular distribution)
Low (0 70)	High (1 18)	Intermediate (1 08)	High-intermediate (1 18–0 93)	Phosphorylase
Low (0 60)	High (1 1)	Intermediate (0 88)	High (0 98)	Glycogen
High (1 08)	Low (0 48)	Intermediate (0 95)	High (1 08)	Esterase
High (1 43)	Low (0.85)	Intermediate (1 32)	High-intermediate (1.38–1.30)	Lipids

and II_i fibers are 40 μm and 39 μm respectively the mean for II_{it} fibers is about 30 μm

M hyoglossus (Data collected from the muscle part running outside the tongue mass) At a fre quency of around 19% group I_m fibers (mean diameter 26 μm) are scarcer than in the other ex trinsic muscles II_m fibers predominate at around 30% (30 μm) while the remaining groups ac counted for 27% (II 43 μm) and 24% (II_i 42 μm) Distributions are scattered

Pars longitudinalis superior m hyoglossi l tal cross sections of the tongue from tip including the entire fiber courses of the ex muscles (i e those parts outside the tongue showed that the thin muscle sheet of long fibers running under the dorsal tongue ep consists entirely of type II fibers The fiber be traced to those parts of the tongue root wh fibers of m hyoglossus pass, but no clusters II fibers could be found there nor were the

Table 2 *Frequency and size of type I and type II fibers in the tongue muscles*

Muscle	Number of fibers	Frequency (%) of fiber types				Mean fiber diameter (μm) and standard dev (Sheppard's correction used)			
		I_m	II	II_{it}	II_m	I_m	II	II_{it}	II_m
Extrinsic									
Genioglossus	1 478	25 4	_3 9	_5 1	25 6	26 9±5 1	39 9±7_	39 4±6.9	30 5±
Hyoglossus	1 713	19 1	26 9	24 1	29 9	26 3±5 0	4 9±7.3	41.8±7 4	29 3
Styloglossus	1 214	22 8	?7 0	22.3	27 9	?9 7±6 4	40 9±7.3	38 7±7 1	31 4±
Intrinsic									
Transversus	506	0.2	36 7	32 4	30 7	–	30.3±4.3	?6.4±3 9	19 9±
Verticalis	531	0 4	10._	6 4	83 0	–	?7.5±6.2	_3 8±5 4	28 2
Pars long sup m hyoglossi	483	–	16 8	66 9	16.3				
Pars long inf m. styloglossi	556	–	14 7	45.8	39.5				

as supported by grants from Karolinska In- ...nder I wish to express my gratitude to Profes- Carlström for kindly allowing the use of the ...ometer The skilful technical assistance of ...e Hoppe is gratefully acknowledged

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tem The superior longitudinal muscle layer is defined as a fiber portion of m hyoglossus It consists wholly of type II fibers running in the dorsal layer most of them originating in the same place as m hyoglossus A similar close connection between the hyoglossal and the longitudinal fibers at the tongue root was earlier reported by Dabelow 1951 (see also Nishi 1938) The peripheral connection found between the superior longitudinal fibers and the fibers of m hyoglossus accords well with reports on the tongue motor nucleus in the central nervous system In their study on dog and rabbit Kosaka & Japita (1903) pointed out that resection of the dorsal tongue half leads to retrograde degeneration of the dorsolateral part of the hypoglossal nucleus an area they and subsequent investigators (Barnard 1940 Morimoto Kato & Kawamura 1966) have shown to comprise the neurons innervating m hyoglossus Further the comparative anatomical study of Hayashimoto (1960) suggested identity between the central projection of the retractive muscles and the innervation area of the superior longitudinal muscle Relatedly Barnard (1940) has suggested that the superior longitudinal muscle splits off from m hyoglossus in embryo

The inferior longitudinal muscle layer is here defined as a fiber portion of m styloglossus composed solely of type II fibers and running in the ventral layer of the tongue Its fiber course differs entirely from various earlier descriptions of m longitudinalis inferior (e g Braus 1924 Abd-El Malek 1938 Cunningham 1964 Gray 1973) Previously the inferior muscle was said to extend from the ventral part of the tongue tip to the hyoid bone in running between m genioglossus and m hyoglossus along the middle part of the tongue The present study reveals that this middle region is composed of a mixture of fibers of types I and II from m genioglossus and m hyoglossus both of which are laterally limited by the type I and II fibers of m styloglossus The lateralmost styloglossal fibers are all type II however forming a fiber portion that can be traced up to the ventral part of the tongue tip and back to the insertion points of m styloglossus The ventrolateral fiber course is quite in agreement with the anatomy of the lateral part of the styloglossal muscle described by Dabelow 1951 M styloglossus receives its central projection from the dorsomedial part of the hypoglossal nucleus (Barnard 1940). The study by Morimoto Kato & Kawamura (1966) suggests that that electrical stimulation of this area leads to retractor[illegible] al deviation of the ipsilateral tongue half [illegible] the tip which becomes ventroflexed.

The assumption that the longitudinal muscle portions are part of the retractive system has further support in the measure[illegible] fiber sizes The longitudinal fibers are [illegible] diameter to the type II fibers of the extr[illegible]cles

Studies of the histochemical properties [illegible] twitch muscles have revealed the presence [illegible] groups of muscle fibers, one of type I, [illegible] type II where all three occur the type [illegible] usually predominates (e g Stein & Padyk[illegible] Henneman & Olson 1965 Kugelberg & E[illegible] 1968 cf also reviews by Close 19[illegible] [illegible] Burke 1973) Recent studies on masticatory[illegible] respiratory muscles confirm this fiber pattern[illegible] Taylor Cody & Bosley 1973 Edström & [illegible] 1973 Edstrom Lindquist & Mårtensson [illegible] also Dubner Sessle & Storey 1978 [illegible] tochemical properties of the tongue muscles [illegible] into this system For instance the fibers [illegible] I_{HB} II_1 and II_{HB} seem comparable to the [illegible] fibers of Stein & Padykula (1962). The II_{HB} [illegible] identified in this study constitutes the [illegible] the hyoglossal and styloglossal fibers [illegible] into the tongue tip

Type II fibers have a dense uniform [illegible] of mitochondria rich in oxidative enzymes [illegible] as a dense peripheral distribution of [illegible] features that are essential structural [illegible] resistance to fatigue (Edström & Kugelberg [illegible] Burke et al 1971) The large amount [illegible] highly oxidative fibers in the tongue musc[illegible] cates considerable fatigue resistance Th[illegible] teristic has in fact been demonstrated in th[illegible] sic muscles which lose only about 20-25[illegible] initial force of their twitch tension during [illegible] activity (Hellstrand 1979)

According to Bárány 1967 and Barnard et [illegible] (see also Burke et al 1971 Kugelberg 197[illegible] related to myofibrillar ATPase activity [illegible] contraction time Since type II fibers [illegible] ATPase activity and type I fibers low the [illegible] muscles might be expected to contract mo[illegible] than the intrinsic which have almost n[illegible] fibers Indeed the mean values of isotonic [illegible] reported for the two muscle groups are abo[illegible] apart the intrinsic muscles being the fas[illegible] (Hellstrand 1979)

...iol Scand 1980 110: 199–201

...pancreatic polypeptide (APP) inhibits atropine ...ant vasodilation in cat submandibular ...ry gland and nasal mucosa ...ble interaction with VIP

... LUNDBERG ANDERS ÄNGGÅRD TOMAS HÖKFELT and JOE KIMMEL

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...pathetic nerve stimulation produces a ...increase in blood flow from the submandibular salivary gland (Bernard 1858) and nasal mu... Änggård 1974) which unlike the secretory ..., is largely atropine resistant (Heidenhain ... Änggård 1974). The submandibular vaso... has been attributed to activation of both ...tor nerves with a special cholinoceptive ...tem (Dale & Gaddum 1930) and metabolic ...on of a kinin system (Hilton & Lewis 1956) ... of kinins has, however been questioned ... et al. 1965). Recent studies indicate the ... of a potent vasodilatory agent, vasoactive ...al polypeptide (VIP), in nerves of both the ...dibular salivary gland (Bryant et al. 1976) ...nasal mucosa (Uddman et al. 1978). The ...res are of parasympathetic origin (Änggård 1979), Lundberg et al. 1980*a*). Further mor... ...ical analysis suggests that VIP is present in ...rgic neurons (Lundberg et al. 1979, 1980*a*). ...le of VIP as a mediator of vasodilation in the ...ndibular salivary gland is further support... ...its atropine resistant vasodilatory action ...zu & Tara 1979, Bloom & Edwards 1980, ...erg et al. 1980*a*). Furthermore, a marked ...se of VIP in the venous effluent from the ...ndibular salivary gland is further support... ...ic nerve stimulation (Bloom & Edwards 1980 ...erg et al. 1980 ...). The vasodilation caused by ...reshold nerve stimulation can partially be ...ed by infusion of VIP antiserum (Lundberg et ...0*a*).

...urther attempts to block the atropine resistant ...dilation, we have focused our interest on avian ...eatic polypeptide (APP). APP is a 36 amino ...polypeptide (Kimmel et al. 1975), which great...ly differs in amino acid sequence from pancreatic polypeptide isolated from other species (Lin & Chance 1974, Kimmel et al. 1975). Recently Lorén et al. (1979) have described the distribution of nerves displaying immunoreactivity to APP in brain and gut. In addition this peptide or a structurally related peptide is present in adrenergic nerves around blood vessels in both the nasal mucosa and submandibular gland of the cat (Lundberg et al. 1980*b*).

Experiments were done on the cat submandibular salivary gland. The systemic blood pressure, local blood flow (via drop counter) and salivation (via drop counter) were recorded on a Grass polygraph. Parasympathetic preganglionic nerve stimulation (6 Hz, 8 V, 1 ms) was performed on the chorda-lingual nerve and sympathetic stimulation on the cervical symphathetic trunc (? Hz, 8 V, 1 ms). Close intra-arterial infusions of APP dissolved in phosphate buffered saline containing 0.5% bovine serum albumin was performed at a rate of 50 µl/min. For details see Lundberg et al. (1980*a*). The nasal blood flow was studied in separate cats. Preganglionic parasympathetic nerve stimulation (parameters as above) was performed on the Vidian nerve in previously sympathectomized cats (Änggård 1974). The cervical sympathetic trunc was stimulated in normal animals (parameters as above). Close intraarterial infusions were done at a rate of 100 µl/min. Atropine (0.5 mg/kg) was given i.v.

Parasympathetic nerve stimulation caused a more than 5-fold increase in submandibular blood flow and a marked salivary secretion (Fig. 1 *a*). Local infusion of APP (0.1 or 1 µg/min) 2 min prior to and during nerve stimulation caused a marked reduction (70±8% ... =4) of the vasodilatory response (Fig. 1...

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-uses a response very similar to the one ly described after infusion of VIP anti- undberg et al. 1980). It is therefore sug- that APP or a structurally similar peptide in- th mechanisms in exocrine glands related Since a close morphological association between VIP and APP-immunoreactive around blood vessels of the nasal mucosa submandibular gland (Lundberg et al. in tion), the inhibitory effect of APP on the atory response may be of physiological ance. The exact (postsynaptic) mechanism inhibition, however, has to be established.

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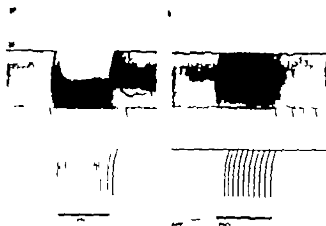


Fig 1 a b Effects of parasympathetic nerve stimulation (6 Hz, 2 min) on cat submandibular gland blood flow and salivary secretion before (a) and after (b) local infusion of APP (0 1 μg/min) BF = blood flow (e ery 10th drop is separately indicated) BP = systemic blood pressure (140 mmHg) S = salivation (every drop is separately indicated) T = time in min

b) Secretion was also reduced to a smaller extent (50±6% *n*=4) (Fig 1 *a b*) 30 min after APP infusion no certain inhibitory effect could be recorded The sympathetic response was not affected by the APP infusion (Fig 2*a* and *b*)

In the nasal mucosa parasympathetic nerve stimulation caused an almost 5-fold increase of local blood flow which was largely atropine resistant Local infusions of APP (0 1 μg/min) reduced this vasodilatory response (80±7% *n*=4) The blockage was reversible The effect of sympathetic nerve stimulation was not influenced by APP

In agreement with previous studies on the submandibular gland VIP (0 1 μg/min) caused a marked vasodilation (Fig 3*a*) This effect was markedly re

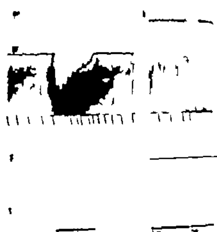


Fig 3 a b Effects of local VIP infusion (0 1 μg ... to and after APP pretreatment (1 μg/min) for ...

duced (90±10% *n*=4) by infusion of APP (... min) prior to and/or during the VIP ... (Fig. 3*b*) On the other hand the effect of ... of acetylcholine (0 1 μg/min) was not ... APP (0 1 and 1 0 μg/min) (Fig. 4a and b)

APP in concentrations up to 100 μg/m ... influence blood flow in salivary gland ... mucosa nor could any effects on systemic ... pressure be recorded

The present findings suggest that APP ... inhibits the nervously induced atropine re... vasodilation both in the submandibular ... gland and the nasal mucosa Also the secre... hibited possibly via an effect on blood flow ... ever interaction with cholinergic mechanism ... not be excluded APP seems to selectively ... the parasympathetic response, since the ... pathetic vasoconstriction was not reduced

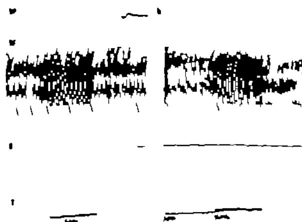


Fig 2 b Effects of sympathetic stimulation (6 Hz, 2 min) on the parameters described in Fig 1

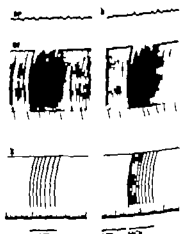


Fig 4 a b Effect of local acetylcholine infusion ... μg/ml) prior to and after APP treatment (1 μg/ min

ol Scand 1980, 110 203–205

nous constriction during cervical sympathetic ation in the cat

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tional importance of the adrenergic in- of cerebral arterial vessels has been the f considerable debate (see Edvinsson & ne 1976) By contrast, the nervous in- n cerebral eins has attracted little atten- ite of the fact that adrenergic nerve termi e been demonstrated in intracranial veins et al 1974) Whereas several reports deal eral diameter changes during sympathetic ion, there is no corresponding study con- cerebral veins In the present study the re- f pial venular vessels to preganglionic cervi- mpathetic stimulation was therefore studied use of intravital microscopy and a multi- l videoangiometer (Auer 1978 Auer & 1979).

experiments were performed in 8 cats with a eight of 1.5–3 kg. The animals were anesthe with 30 mg kg sodium pentobarbital im- ted with 6 µg kg pancuronium-bromide ed endotracheally and respirated with a 3 1 e of N_2O O_2. Body temperature was con sly controlled with Philips rectal thermo- ent and maintained at 37°C 38°C by aid of a g pad. Mean arterial pressure was continuous- itored via a PVC catheter inserted into the ding aorta through the left femoral artery moral vein was cannulated for the eventual stration of bicarbonate solution in order ntain acid-base balance. The right femoral ar as cannulated for frequent blood gas analysis gas check). The right cervical sympathetic was isolated and sectioned below the superior thetic ganglion and the cranial end mounted olar silver electrodes fixed into a plastic tube vertical were then brought into a prone posi- and the head was fixed in a stereotaxic head- r A parietal cranial window with diameter mm ipsilateral to the sympathetic preparation was made closed with a glass shield and sealed with acrylic (Auer 1978).

Pial venular vessels were investigated through a Leitz intravital microscope and the data were eval- uated by aid of a multichannel video-angiometer (Auer & Haydn 1979) Single experiments were monitored with a TV-system and stored on video tape Measuring lines—freely movable on the TV screen—could then be placed on any wanted vessel portion to obtain continuous data concerning the vessel diameter variations expressed in micrometer (µm). Multiple replay of experiments on tape thus allowed analysis of al arge number of vessel seg- ments. The sympathetic nerve was stimulated for 90 s with square wave pulses of 10 ms duration at 15 Hz so as to produce maximal pupil dilatation and retraction of the membrana nictitans (1 10 V).

The resting mean arterial pressure was 118±7 mmHg (SE). No significant change was noted dur ing sympathetic stimulation $PaCO_2$ remained con- stant at 29 6±0 4 mmHg and PaO_2 at 101±3 mmHg throughout the experiments Continuous record ings of venous caliber changes were performed from 103 vessel segments with resting diameters be tween 32 and 486 µm mean essel calibre 141 ± 9 4 µm. Pial veins started to constrict 9 1±1 s af ter the onset of stimulation. Maximal constriction was in most instances reached at 90 s or even some what later when stimulation had already been dis- continued. However in 11 vessel segments maxi- mal constriction was observed after 40-80 s. In 5 vessel portions a slight dilatation was observed (see Fig. 1). No consistent tendency could be demon- strated when the time course of reactions of smaller and larger veins was compared at the highest paper speed of a multichannel penwriter Statistical com- parison between vessels with diameters up to 150 µm and those above 150 µm showed no significant difference of the time reaction pattern: veins ≤150

r of veins during sympathetic stimulation comment to a figure by Forbes (1928) vein did not show measurable change nction of larger veins obtained in the pres- of about 20% would have an important on the blood volume The physiological ce of the present findings remains to be ring spontaneous alterations of the sym- nervous activity during physiological as athological conditions However the pres- ts show that possiblity for changes in cere d volume by alterations in sympathetic ac at hand which is in agreement with histo- ?] studies (Owman et al. 1974)

y as supported by the "Österreichischer Fonds schaftlichen Forschung the Swedish Medical Council (projects No 14X-4968) and the Swed- al Association against Heart and Chest Diseases

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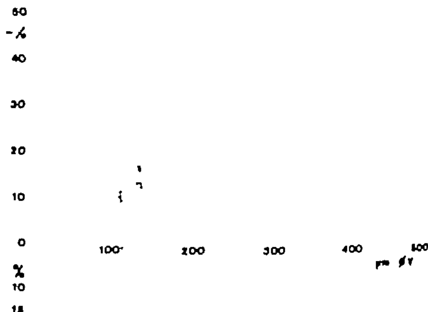


Fig 1 Percentual pial venoconstriction during sympathetic stimulation plotted against resting caliber Single smaller branches show slight dilatation but basically there is a clear tendency to marked vasoconstriction with veins >150 μm resting diameter reacting stronger than smaller ones.

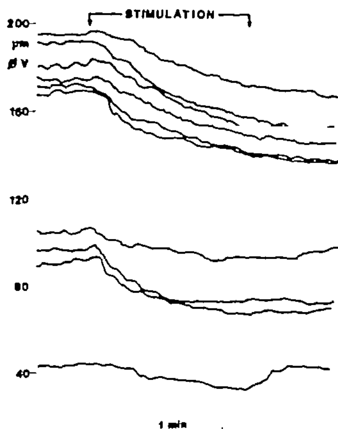


Fig 2 Multichannel videoangiometric recordings of pial venous reaction during sympathetic stimulation. Venous diameter is given on the ordinate

μm resting diameter started to constrict 10.8±1 6 s those >150 μm within 7.2±0.1 s

Veins with a resting diameter >150 μm constricted more than smaller veins The percentual constriction is plotted against resting diameter. Mean constriction of veins ≤ 150 μm resting diameter was 11.5±0 9% and of veins >150 μm ... 1 9% (Student s *t* test P<0 0005 for both) when compared to resting levels. Typical examples of diameter changes during sympathetic stimulation are shown in Fig 2

Considering the importance of blood volume for the intracranial pressure and the fact that the venous compartment contains about 80% of the global blood volume it is surprising that so little attention has been given to the regulation of cerebral veins The tone of the veins is mainly regulated by sympathetic nervous activity in other vascular beds (Mellander & Johansson 1968) Sympathetic stimulation has been shown to reduce the cerebral blood volume in mice (Edvinsson et al 1971). The sympathetic influence on intracranial pressure is well documented an effect that could be mediated via changes in blood volume and/or via alterations in the secretion of cerebrospinal fluid (Edvinsson et al 1980)

The only previou ... ion we have for

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Ine sensitive contractile motor effects ıstance P on the feline pylorus tomach in vivo

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ls Motility stomach, pylorus, substance P and cat

ıence of gut stimulating depressor sub- n intestine and brain, later called Substance was originally reported by Euler & Gaddum The contraction of intestinal smooth muscle e distinguished from the action of ACh as not inhibited by atropine in vitro The actor was later demonstrated to be an 11 acid peptide (Chang et al 1971) identical with n, which caused an atropine-resistant in- of salivation in vivo in rats (Leeman & ecradling 1967).

s widely distributed throughout the body ılarly in the central nervous system and the enow 1953). By the use of immunohisto- ıtry SP was localized not only to various of the brain but also to primary sensory s at the spinal ganglia, the dorsal root and rsal horn of the spinal grey matter (Hökfelt et 77). In the mammalian gut, the highest con- tions were found within the duodenal and walls, where SP in some species was local- ı populations of serotonin-containing entero- taffin cells (Pearse & Polak 1975). By immuno- hemistry SP was also demonstrated within cell bodies and terminals of the intramural s (Schultzberg et al 1980).

has been regarded as tentative neurotrans- but in several biological test systems this has questioned because of the slow onset and long ion of it effects (cf Polak & Bloom 1979) The rence of SP in dorsal roots and spinal cord has the assumption of SP as a central excitatory mitter of some primary afferent neurons sup- d by the electrophysiological findings of Ot- suka et al (1975). The functional importance of SP in the peripheral nervous system has been focused on antidromic vasodilatation (cf Lembeck et al 1979), but the localization of SP in nerve terminals not only around blood vessels but also along smooth muscle cells and in intramural ganglia of the gut (Hökfelt et al. 1977) may indicate other func tions as well (Katayama et al 1979) Immuno- histochemical studies in the cat further suggest a more dense SP innervation of the gastrointestinal sphincters than of other gut regions (Lundberg et al. 1980)

The purpose of the present study was to record the motor effects of local i.a. administration of SP on the feline pylorus and stomach of anesthetized cats in vivo.

11 cats in chloralose anesthesia (50 mg/kg b.w.) were used. Intraarterial blood pressure and gastric and pyloric motility were continuously recorded. Gastric motility was studied by volume recordings of flaccid intragastric balloon at constant intra- luminal pressure (Martinson 1965) Pyloric motility was recorded as changes of an applied constant flow of body warm saline across the sphincter (Edin 1980). A fine heparinized plastic catheter (Ø1 mm) was introduced towards the antro-pyloric region via the splenic artery in retrograde direction Ink in- fusions after the experiments demonstrated a re gional distribution to the upper part of the duo- denum, the pylorus and the gastric antrum-corpus. SP (Peninsula, Ca. USA) was dissolved in saline and given as a single bolus dose of 0.5–1 ml volume (0.5–4 μg) via this catheter SP injected i.a. induced prompt powerful contraction of the tomach and

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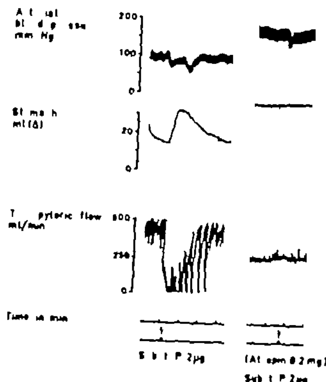


Fig 1 The effects of regional I a. injection of SP on intra-arterial blood pressure gastric motility and transpyloric flow in the anesthetized cat before and after the administration of atropine I v After atropine the effect on blood pressure was not completely blocked while both motor effects were totally abolished

the pylorus of ?–4 min duration in all animals A slight reduction of the blood pressure of similar duration was also obtained (Fig 1) The duration of these effects was dose-dependent In the pylorus even the low doses caused a total but comparatively short cessation of the transpyloric flow initially No gradation of the gastric contractile response was demonstrated However only the four doses 0 5 1 2 and 4 μg of SP were tested in one and the same animal 10 cats were given atropine (0 mg/kg I v) before SP was tested again The effects on motility caused by SP were blocked in 9 of these 10 atropine pretreated cats (Fig 1) while it induced a short lasting (<1 min) 50 % decrease but no cessation of the transpyloric flow in one animal However the SP induced decrease in blood pressure was not totally blocked by atropine (Fig 1) It has earlier been demonstrated that this dose of atropine does not block the pyloric motor responses obtained after enkephalin and VIP I a (Edin 1980) The specificity of the atropine blockade was demonstrated by I a injections of ACh (100 μg) After atropine the ACh induced contractions were totally abolished

Thus the present study demonstrates ... administrated via a regional artery ... powerful contractions of the stomach and ... rus of a few minutes duration Since SP ... munoreactivity has been demonstrated ... ganglion cells of the two intramural plexuses ... surrounding nerve terminals of the pylorus ... as of the stomach the possibility of SP a ... neurotransmitter in the gut should be ... Furthermore axons in the vagal nerve ... like immunoreactivity demonstrated ... the nerve at the abdominal level (Lundberg ... 1980) These fibres may originate at least ... from cell bodies of the nodose ganglion ... strated to contain many SP-positive ... (Lundberg pers comm.). Surprisingly ... these experiments the motor effects of SP ... blocked by atropine in contrast to most ... vitro studies This suggests that the contra... tor effects of SP observed in this experim... vivo model probably involves the activation ... citatory cholinergic neurons presumably ... the intramural plexuses In the living animal ... effect by SP may either be accomplished ... mural SP neurons or via sensory SP fibres ... vagal or splanchnic nerves (cf Lundberg ... 1980) Since the vagally induced pyloric con... earlier studied by this model is atropine ... but sensitive to hexamethonium and naloxone ... 1980) vagal "sensory SP fibres seem less ... mediate the effect observed in this study

This study was supported by grants from Gö... Läkaresällskap the Medical Faculty of Göteborg ... Swedish Medical Research Council (17X 5... 04P-4173)

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...ensitivity of perifused fat cells to the antilipolytic ...n of adenosine

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...ne may be an important modulator of lipo... adipose tissue (cf Schwabe et al. 1975 ... m 1978) Adenosine accumulates during in... of adipocytes and is potent inhibitor f ...MP accumulation in isolated fat cells (Fain ...72, Ebert & Schwabe 1973 Stock & Prilop ...jerndahl & Fredholm 1976) The accumula... modulating substrates such as adenosine in ...nity of the fat cells is prevented by peri... of fat cells (Allen 1973) since the cell are ...ously exposed to fresh perfusate The anti... effect of adenosine on fat cells has been ...strated ...th this technique (Turpin et al ...ljerndahl & Sollevi 1978) However the anti...c potency of adenosine was more than 100-...gher in the work by T...rpin than in our study ... Turpin et al (1977) reported that a 10 nM ...tration of adenosine reduced a submaximal ...c response to adrenaline by 50% whereas we ...ound a 25% inhibition by 1 μM adenosine ... noradrenaline as the agonist. Basal plasma ...sine levels are close to 0 1 μM (Fredholm & ... 1980). The results of Turpin et al (1977) ...mply an almost maximal effect already under ... conditions, while our results suggest that the ... of adenosine have to be raised above basal to ...ce an antilipolytic effect To understand the ...f adenosine in the regulation of lipolysis it is ...ore important to clarify which of the estimates ...tilipolytic potency is correct

...ymal fat ...as obtained from decapitated male Spra... ...wley rat (Anticimex) weighing between 150–450 g ... 200 g) Isolated fat cells ...ere prepared, using 3 mg/... crude bacterial collagenase (Worthington Biochem... ...) After the final ...ashing 1 ml aliquot of packed ...as transferred to ...temperature-controlled plastic ...ber containing 2-2 6 ml Krebs-Ringer phosphate ... pH 7 4 ...th half the recommended calcium con...ation, 5 5 mM glucose and 1% bovine serum albumin ...ma, St Louis) The floating fat cells were sub...sequently "perifused" (Allen et al. 1973) with this buffer at 37°C and rate of 2 ml/min. Drugs were infused via side-arm before the chamber Lipolysis was measured as glycerol production rate (nmoles ml cells^{-1} min^{-1}) Glycerol was measured by the method of Laurell & Tibbling (1966), but the $ZnSO_4$–$Ba(OH)_2$ precipitation step was eliminated as in the previous study (Hjemdahl & Sollevi 1978) to increase the sensitivity Standard curves for glycerol in the perifusion buffer gave expected values despite this modification The glycerol assay was not influenced by the drugs administered.

The perfusates were collected in ice-cooled plastic tubes and immediately frozen thereafter When thawed the samples (25–100 μl) were immediately and without purification analyzed for adenosine and inosine by high performance liquid chromatography A Waters model 6000A solvent delivery system and Waters U6K injector was used The separation was performed on Waters μ-Bondapac C_{18}-column protected with 40 mm precolumn packed with the same material. The mobile phase (0 01 M NH_4PO pH 6 0 containing 14% methanol was delivered at 1 5 ml/min). Absorbance was continuously monitored at 254 nM by Waters model 440 absorbance detector and recorded on Kipp and Zonen recorder Peak heights in these chromatograms are linearly related to the amount of adenosine inosine and other purines over wide range (2–100 pmol) and were therefore used for quantification

There was basal outflow of glycerol from the isolated perifused fat cells of 8.3±1 8 nmol ml cells^{-1} min (n=12) Adenosine and inosine concentrations in the perfusate were below the detection limit (5–10 nM) during both basal and stimulated conditions, indicating a very low adenosine level in the medium surrounding the cells. In keeping with the concentration of adenosine required to inhibit lipolysis and the adenosine levels found in the perfusat of our system, we previously have not (Hjemdahl & Sollevi 1978) found that adenosine deaminase (ADA) enhances lipolysis under these conditions During adenosine infusion (1 μM) glycerol outflow was 7.3± nmol ml cells min (n=7) which was not significantly different from the basal lipolysis (see also Turpin et al. 1977)

...ic effect of adenosine would have to be ...rned by mechanisms keeping the adenosine ...ch lower than those commonly encoun... ...ssues and body fluids. Such a high sensi... for example not compatible with basal ...vels in the 10^{-7} M range since that would ...e fat cells virtually unresponsive to ad... stimulation which increases the levels of ...e to the µmolar range. On the other hand ...ent results confirm our previous estimate ... lipolytic effects of adenosine require adeno... ...centrations in the µmolar range. Under ...ditions adenosine levels are probably too ...gnificantly affect lipolysis and only when ... raised by drugs or by adrenergic stimula... ...redholm & Sollevi 1977, Fredholm & Sollevi ...redholm & Sollevi, in manuscript) are anti... ... concentrations reached.

...y ... as supported by the S...edish Medical Re... ...uncil (proj. no. 04X 2553)) and by Karolinska...

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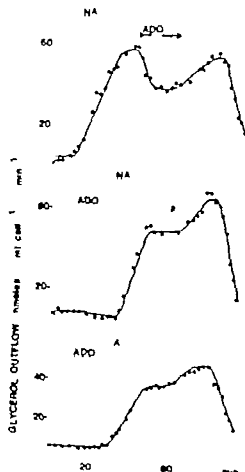


Fig 1 Three typical experiments showing the antilipolytic effect of 1 μM adenosine (ADO) in perifused rat fat cells In the upper figure adenosine was introduced during stimulation with noradrenaline (NA 0 13 μM) and caused 31±3 (n=5) per cent inhibition of glycerol outflow In the middle figure adenosine was introduced prior to stimulation thereby inhibition glycerol outflow by 25±5 (n=4) per cent When adrenaline (A 0.3 μM) was the stimulating agent (lower figure) adenosine inhibited glycerol outflow similarly by 4±3 (n=3) per cent

A submaximally effective concentration of adrenaline (0 3 μM) which was used as lipolytic stimulus by Turpin et al (1977) was equally potent as noradrenaline (0.3 μM) in stimulating lipolysis in our system the glycerol production rate being 33±5 (n=3) vs 36±4 (n=9) nmol ml cells min respectively Noradrenaline and adrenaline stimulated lipolysis was equally affected by the addition adenosine and the inhibition produced was of the same order of magnitude as that reported previously by us (Fig 1) (Hjelmdahl & Sollevi 1978) Thus the difference in choice of lipolytic agent can not explain the marked difference in antilipolytic potency of adenosine found in the two conflicting studies on perifused fat cells (Turpin et al 1977

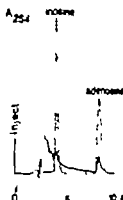


Fig 2 A chromatogram showing the presence of ... sine and inosine in the effluent perfusate obtained ... adenosine infusion—solid line The dotted ... gram represents a standard solution containing adenosine and inosine respectively which was ... as the sample The concentration in the sample were 0 8 μM adenosine and 0 2 μM inosine

Hjemdahl & Sollevi 1978). Another differ... tween these studies was the order in which ... sine and lipolytic agent were introduced ... in our hands adenosine was equally potent ... iting lipolysis whether introduced before ... stimulation (Fig. 1).

It is known that bovine albumin is cont... with various amounts of adenosine de... (ADA) capable of degrading exogenously a... tered adenosine Fain & Malbon (1979) s... that the less pronounced antilipolytic ... adenosine found by us (Hjemdahl & Sollev... could be due to extensive degradation of ad... by ADA activity in the albumin contain... fusates This degradation was supposed to ... effective adenosine concentration in the ... ing of the fat cells in the same range as the ... Turpin et al (1977) However determin... the adenosine and inosine concentrations ... effluent perfusate during adenosine infusion ... gave values of 0 8±0 1 and 0 2±0 1 μM ... respectively indicating degradation of le... 25% of the adenosine under the present cond... A typical chromatogram from one of the... periments is shown in Fig 2

Thus the present results do not offer an ... planation for the very high antilipolytic pot... adenosine reported by Turpin et al (1977). P... the strain of rats used by these authors is ... sensitive However ... igh sensitivi...

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turally reduced compliance of the venous capacitance ls in spontaneously hypertensive rats (SHR)

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ence of cardiac arterial and arteriolar wall phy in primary hypertension was recog- er a century ago though the important namic consequences were realized only re :f Folkow 1978) Far les is known about pressure capacitance sections, where simi ctural adaptation to pressure alterations occur albeit with different hemodynamic ences Thus, while media hypertrophy for ic reasons amplifies precapillary resistance es, such consequences are negligible in the thinwalled capacitance vessels Instead ickening should her mainly increase con- strength and/or reduce wall compliance th important consequences for cardiac and overall hemodynamics

ase of local load and muscle activity—the stimuli for wall hypertrophy—often seem to also in the low-pressure cardiovascular sec primary hypertension For example the ac ted autonomic in ol ement in neurogenic includes venoconstriction causing blood centralization and pressure elevation in l low-pressure parts (Julius & Esler 1975) hen left entricular hypertrophy develops alls for an increased filling pressure (cf 1978) Furthermore even distal venous res seem to be elevated to judge from signs dest capillary pressure elevation (Ulrych 1979 Lundin & Folkow 1978)

cerning man, both olume (Ulrych 1979) and smographic studies (Takeshita & Mark 1979) st a reduced enous distensibility in primary tension, present also after constrictor fibre ade Though a tructural compliance reduc likely to contribute it is in man difficult clude non-neurogenic increases of venous th muscle tone In rat primary hypertension complete vascular relaxation is easy to en- Simon s findings (1976) suggest structurally reduced venous compliance Here however the nitial enous volume was not known, which is nec essary for estimations of wall compliance, and the retrograde filling technique used might have un predictably delimited the venous segments dis- tended because of distal valves

In this laboratory the relationships between al- tered cardiovascular design and hemodynamics in normotension and hypertension has been much studied (Folkow 1978) Particularly the rat hind quarter vascular bed has here been used for estima- tions of pre and postcapillary resistances capillary permeability and capillary volume (Kamiya, Rippe & Folkow 1979) It is therefore a suitable target for precise estimations of venous volume and wall dis- tensibility as well

pro-

The experimental approach was based on the isogravi- metric technique described elsewhere (for ref see Folkow 1978). Briefly isolated hindquarters from 10 pairs of matched spontaneously hypertensive rats (SHR) and Wis tar normotensive control rat (NCR) (6–7 months of age differing about 50% in mean arterial pressure) were per fused with constant flow at papaverine-induced maximal vasodilatation during continuous measurements of tissue weight and of inflow and outflow pressures (P and P) They were subsequently used also for other purposes I the initial control situation P and P were set to iso- gravimetry but with P at 4 mm Hg to avoid both venous semicollapse and true wall distension. A the isogravi- metric capillary pressure (P_{ci}) can be measured as well, the control pressures at both ends of the venous capaci- tance section (P and P) could be defined. I identical hindquarter preparations total intravascular volume pres- ent in this control situation, was deduced from flow and mean transit time (cardiogreen) data.

From this control level known P elevations (and hence known P elevations) were induced during constant flow with measurements of the increased intravascular con- tents as derived from the initial rapid slope of weight in- crease Thus the relationships between average venous pressure increment

$$\Delta\bar{P} = \Delta\left(\frac{P_{T}\;P_{T}}{2}\right)$$

protein escape in SHR (Rippe Lundin & 978) and hypertensi e man (Ulrych 1979) suggests a modest elevation also of average pressure in both types of primary hyper

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and intravascular volume increase could be estimated with considerable accuracy over the (for rats) physiological $\bar{P}_v$ range about 6–20 mmHg

Results The study has allowed deduction of average venous compliance in SHR and NCR In each group uniform results were obtained showing an almost linear relationship over the mentioned $\bar{P}_v$ range between increases of $\bar{P}_v$ and intravascular volume both in SHR and NCR Between SHR and NCR there was however a clearcut difference since the intravascular volume increase/mmHg $\bar{P}_v$ elevation ×100 g tissue was 0 101±0 005 (SE) in NCR but only 0 083±0 004 in SHR at complete vascular relaxation This 20% difference tended if anything to become reduced when graded noradrenaline vasoconstrictions were induced which caused largely equal increases of $\bar{P}_v$ and venous resistance in SHR and NCR but in SHR the characteristic structural accentuation of precapillary resistance increase (Folkow 1978) It should here be noted however that venous resistance and capacitance changes are not necessarily congruent since e g venular sections may contribute far more to resistance than to capacitance

To estimate average venous distensibility also the initial absolute ('control') venous volumes must be defined at least in approximate but for NCR and SHR equivalent figures They can only be deduced indirectly though with reasonably small error from total hindquarter intravascular volumes if corrected for precapillary and capillary volumes Flow and mean transit time measurements gave control intravascular volumes of 5 5±0 2 ml/100 g hindquarters being if anything, slightly larger in SHR than in NCR

In most vascular beds the precapillary section contains some 20% of total contents and the hindquarter exchange vessels very nearly 10% (Kamiya Rippe & Folkow 1979) leaving about 70% for the venous compartment (cf Alexander 1963) There is no reason to believe that SHR and NCR differ appreciably concerning the small capillary exchange volume but the 40–50% higher precapillary resistance in SHR (Folkow 1978) implies a 20% smaller microvascular precapillary volume though offset by usually slightly wider large arteries in SHR Anyway with only 20% total precapillary volume a minor SHR NCR difference hardly affects the deduced 'control' venous volumes which would then be about 3 8 ml/100 g hindquarter in both NCR and SHR. —As the experimental $\bar{P}_v$ elevations raise also precapillary pressure though to a lesser degree, precapillary [illegible] contributes to the volume increments [illegible] since the precapillary volume and pressure [illegible] much smaller and arterial walls far stiffer [illegible] 90% of the volume increases to $\bar{P}_v$ elevation reflect venous distension (Alexander 1963)

Conclusion and comments The combined 20% reduction of venous distension in SHR [illegible] anything a slightly larger 'control' venous [illegible] suggests that the difference between SHR [illegible] is not merely one of 'unstressed' hindquarter venous volume It rather reflects a true reduction [illegible] hindquarter venous wall compliance, which must reflect a structural stiffening and/or [illegible] of the venous non-contractile wall [illegible] SHR Expressing venous compliance as per [illegible] volume increase per mmHg increment of [illegible] venous pressure ($\bar{P}_v$) the figure for the NCR venous capacitance side is about [illegible] 2 0% for SHR Estimations of venous [illegible] during noradrenaline vasoconstriction [illegible] difficult to quantify as venous distension [illegible] reduced but also in this situation the SHR [illegible] tance vessels seemed to be modestly stiffer [illegible] differences in venous wall compliance [illegible] small but hemodynamically they can be [illegible] portant for volume distribution, capillary [illegible] cardiac filling pressures in hypertension.

In general these signs of a structurally [illegible] compliance of the venous capacitance [illegible] SHR hindquarters agree well with recent [illegible] metric data from isolated SHR veins by Greenberg [illegible] Palmer & Wilborn (1978) However here [illegible] sence of a significant venous pressure [illegible] the acute measurements led to the interpretation that venous wall hypertrophy may be [illegible] independent in SHR Even though also other [illegible] ings suggest a genetic facilitation of structural [illegible] diovascular adaptation in SHR (Folkow 197[illegible] lationships between load and tissue design [illegible] judged on the basis of average pressures over [illegible] periods particularly for veins The reason [illegible] wide fluctuations in both venous pressure [illegible] neurogenic venous engagement during daily [illegible] For example if the daily average for cardiac [illegible] sure rose from 2 to 2 5 mmHg an increase [illegible] ficult to ascertain since fluctuations around [illegible] mean are much larger this would be enough [illegible] 25% wall thickeni[illegible] fact the increased [illegible]

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fication of 15-hydroxy 5,8 11 13-eicosatetraenoic 15-HETE) as a major metabolite chidonic acid in human lung

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mation of arachidonic acid in guinea pig been shown to afford 1 HETE by a l p- se pathw y and to a considerably greater XA as well as 12 HHT by the fatty acid /genase pathway In addition, small of 11 HETE and 15-HETE (1.6 and 0.. % ered products respectively) were isolated rg & Samuelsson 1974) The corresponding f arachidonic acid transformation n human not known. However it might be expected fferent since chopped human lung has been d to release PGE_2 and $PGF_{2\alpha}$ (analysis by y or radioimmunoassay) (Piper & Walker Jathé 1976)

e present communication we wish to report nsformation of arachidonic acid in human with special reference to the pattern in asth- ung tissue. The results show an extensive sion into C_{20} monohydroxy acids, notably TE, in asthmatic lung, and similar but less l conversion in non-asthmatic lung.

rods Lung specimens were obtained from 6 of either sex undergoing pulmonary sur Four of the patients were non-asthmatics as the remaining two had long history of and asthma (type I allergy) All patients were the same premedication and anesthesia, the atics in addition terbutaline At the time of) one of the asthmatic patients showed d and spirometric reversibility of broncho- iction on inhalation of isoproterenol, whilst her asthmatic was almost symptom-free and ormal spirometric values possibly as a result cessful hyposensibilization The lung sample this latter patient converted arachidonic acid same rate as those from the non-asthmatics. s of peripheral and macroscopically fresh lung) (3–4 g) were chilled and rinsed in ice-cold Tyrode solution immediately after the resection The tissue was subsequently m nced and homo- genized in 3 volumes of potassium phosphate buffer (0 1 M pH 7 4) containing 10–100 µg of (1 ⁴C)- arachidonic acid The homogenates were shaken aerobically at 37° for 30 m n Ethanol was added and the incubation mixtures were extracted twice with diethyl ether at pH 3 The residue obtained after evaporation of the sol ent was treated with diazomethane and subsequently subjected to thin layer radiochromatography The solvent systems used were the organic layer of ethyl acetate–2,2,4- trimethylpentane–water (50 100 100 /v/ anal- ysis of monohydro y esters) and methanol–diethyl ether (2 98, /v analysi of methyl esters of prosta- glandins and TXB_2)

Result Incubation of [1 ⁴C]arachidonic acid with 1 asthmatic and 4 non-asthmatic lungs re sulted in a low (3–6%) conversion into mono- hydroxy acid(s) as judged by thin layer radiochro- matography The R_f value of the peak of mono- hydroxy acid(s) (R_f=0 52) was the same as the R_f values of 11 12 and 15-HETE, but different from that of 12 HHT (R_f=0 45) It thus seemed likely that the hydroxy acid peak was due to C_{20} mono- hydroxy acid(s) but the composition of this materi- al was not studied further Thin layer radiochro- matographic analysis using a more polar solvent system revealed a peak of variable intensity at the origin, corresponding to phospholipid-bound [1 ⁴C]arachidonic acid. In one out of the four non- asthmatic lungs there was in addition a trace of ra- dioactivity associated with the reference spot of PGE_2. Formation of $PGF_{2\alpha}$ PGD_2, 6-keto- $PGF_{1\alpha}$ or TXB_2 was not observed

Incubation of [1 ⁴C]arachidonic acid with homo- genate of the other asthmatic lung led to substantial (25%) conversion into material migrating only like

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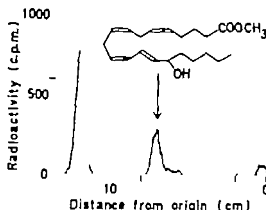


Fig 1 Thin layer radiochromatogram of esterfied product obtained following incubation (37° 30 min) of 100 μg [1 ¹⁴C]arachidonic acid with a whole homogenate of 3 g of asthmatic lung in 9 ml of 0 1 M potassium phosphate buffer pH 7 4 The solvent system was the organic layer of ethyl acetate–… 4-trimethylpentane water 50 100 100 (v/v/v)

C_{20} monohydroxy acids (R_f = 0 5?) (Fig 1) Part of the material was converted into the trimethylsilyl ether derivatives and analyzed by mass spectrometry The mass spectrum showed ions of high intensity at m/e 406 (M) 335 (M 71) loss of $(CH_2)_4$-CH_3) 316 (M 90 loss of trimethylsilanol) 225 ([CH=CH–CH=CH–CH (OTMSi)-$(CH_2)_4$-CH_3]) and 173 (TMSi-O =CH-$(CH_2)_4$-CH_3) indicating that the parent acid was 15 hydroxy 5,8 11 13-eicosatetraenoic acid (15-HETE) However the mass spectrum also showed weak ions indicating the presence of small amounts of 11 HETE and 1 HETE In order to determine the percentage composition of the C_{20} monohydroxy acids the material was subjected to catalytic hydrogenation converted into the TMSi ether derivatives and analyzed by mass spectrometry Several spectra were recorded on the hydroxy acid peak as were spectra on the references the TMSi derivative of methyl 11 12 and 15-hydroxyeicosanoates The references gave each two ions of high intensity i e m/e 229 and 87 (11 hydroxy) m/e 215 and 301 (12-hydroxy) and m/e 173 and 343 (15-hydroxy) The ions mentioned were present in the sample showing the following percentage composition of hydroxy acid isomers 15-HETE (89%) 1 HETE (9%) and 11 HETE (2%)

Discussion $PGF_{2\alpha}$ is a potent bronchoconstrictor in particular in patients with bronchial asthma (Mathé et al 1973) and the plasma levels of 15 keto-13 14-dihydro-$PGF_{2\alpha}$ (metabolite of $PGF_{2\alpha}$) increase markedly when such patients are provoked with specific allergen (Green, Hedqvist & S 1974) As a consequence $PGF_{2\alpha}$ has bee ered to have a mediator role in bronchial The present results do not necessarily rule a possibility but they indicate that transfor of arachidonic acid by lipoxygenase rather t cyclooxygenase seems to be the preferred p in human lung parenchyma Thus the tissue showing the highest conversion of arachido (25%) and hence offering the best chance to formed products was found to give rise mainly to the C_{20} monohydroxy acids 15-HETE (8 HETE (2%) and 11 HETE (1%) of which HETE can be formed by the cyclooxygenase tem It may be noted that this tissue obtained from a patient with bronchial asthma same radiochromatographic pattern was seen remaining 5 experiments although due to l conversion rate the material could not be a lyzed by masspectrometry In one of the asthmatic lungs there was an additional radioactivity having the same R_f value as P is suggested therefore that 15-HETE is the derivative of arachidonic acid in asthmatic and asthmatic lung

Presently it is not known whether 15-HE se has any pulmonary effects However mediate precursor 15-HPETE is a well k hibitor of prostacyclin ynthetase (Moncada 1976) Prostacyclin is a potent vasodilator been reported to reduce unspecifically bronchoconstriction in man (cf Moncada & 1979) Since prostacyclin is released from lungs presumably from vascular endothelial in several animal species it is at least theor possible that inhibition of prostacyclin format 15-HPETE could alter the perfusion-ventilation lationships in the human lung

In the present study there was no indicat formation of 5-HPETE parent substance of the monary very active leucotrienes (Samuelsson 1980 Hedqvist et al 1980) It should be k mind however that 15-HPETE and 5-HPETE structurally very similar and that they thus might give rise to compounds having similar Experiments are in progress to investigate interesting possibility

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oceptive influence from trunk region onosynaptic hind limb reflexes in the cat

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vious investigation (Carlson & Lindquist was shown that stimulation of the trunk skin ve in eliciting reflexes in the lumbar back Nociceptive stimulation of the dorsal as the trunk evokes reflex activity in back of both sides whereas stimulation applied at skin regions located ventrally may inhibit tonic muscle activity Effects of limb skin ion upon back muscle reflex acti ity were served, indicating the presence of reflex ions controlling the activity in trunk mus- ing withdrawal movements of the limbs.

im of the present series of experiments was ine the possible influence of trunk skin af on proprioceptive reflexes of hindlimb mus- adult cats were used for the experiments. esia as initiated by ether and in 4 cats ned by i. administration of chloralose mg per kg b wt) In 5 animals the spinal cord transected at the C level after ligation of the ls The animals were curarized by injec of Flaxedil and ventilated artificially ectomy (L_4–L_7) was performed on 6 cats. nerves to flexor digitorum brevis (FDB) and (FDL), gastrocnemiu laterals and medialis nd GM), tibalis anterior and extensor di- brevis (peroneus PER) as well as skin and entral and dorsal root were prepared cording and stimulation. Monosynaptic re were elicited either by stimulation of dorsal and recording from muscle nerves or by ation of muscle nerves and recording from l roots, as indicated in Fig 1 These test es ere conditioned by mechanical stimula- f the trunk skin (pinch with a pair of forceps) electrical timulation of different trunk skin (square wave pulses of 0 1–0 4 ms duration rengths up to 15 times threshold)

of mechanical trunk kin stimulation tatory as well as inhibitory effects on test re could be demonstrated by pinch stimulation d to the skin Light touch or pressure was ctiv In GM inhibition was regularly seen when stimulating the skin covering the tail and the dorsal side of the trunk up to a level corresponding to the 3rd lumbar ertebra. The inhibitory effect was most pronounced by pinching at the lower lumbar levels and gradually decreased at higher level Inhibition was also observed when stimulat ing the contralateral skin areas. Similar effects were observed on test reflexes in FDB and FDL, supply ing toe flexors In PER, which innervates ankle flexors and toe extensors, no clear effects of dorsal skin stimulation could be demonstrated. Pinch stimulation applied to entral skin areas on the trunk did only occasionally change the test reflexes in the different muscle nerves. In some experi- ments however facilitation of the test reflex in PER occurred. From these observations it is con- cluded that trunk skin nociceptive afferents ha e connections with motoneurons supplying muscles acting at the ankle and in the foot

Effects of electrical trunk skin nerve stimulation The nervous connections between the trunk skin afferents and the limb muscle motoneurons were further studied in a series of conditioning test reflex experiments using electrical stimulation of skin nerves. Stimulation at varying intensities was applied to cutaneous nerve branches from the dorsal and ventral rami of the spinal nerves supplying the dorsal and ventral aspects of the trunk respectively

In most of the nerves tested (GM GL, FDB and FDL) the monosynaptic reflexes were usually inhib- ited by the conditioning volleys in the dorsal skin nerves (see figure). The inh bition was observed at test-conditioning intervals ranging from 15 up to 40 ms and was most prom nent at the longer intervals. In PER, however facilitation was generally ob- served as exemplified in the uppermost pair of records. The small monosynaptic test reflex (first peak in left-hand record) showed 3-fold increase in amplitude after conditioning (second peak in right-hand record) In this experiment also a mul- tisynaptic olley appeared which likewise showed some facilitation. The volley preceding the con-

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·Ischemic coma in rat
t of different pre-ischemic blood glucose levels
erebral metabolic recovery after ischemia[1]

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SIEMKOWICZ, E. & GJEDDE, A.. Post-ischemic coma in rat: Effect of different pre-ischemic blood glucose levels on cerebral metabolic recovery after ischemia. Acta Physiol Scand 1980, 110: 225–232. Received 2 Aug. 1979. ISSN-0001-6772. Departments of Anesthesia, University Hospitals (Hvidovre) and Medical Physiology A, The Panum Institute, Copenhagen University Denmark.

Hyper- normo- and hypoglycemic rats were exposed to 10 min of complete cerebral ischemia. Regional cerebral blood flow (CBF), blood-brain glucose transfer and cerebral consumption of oxygen and glucose were measured before as well as three and 60 minutes after ischemia. Three min after ischemia, no differences were observed between the 3 groups of rats. One h after ischemia, the hyperglycemic rats in comparison to those of the other groups had similar whole-brain CBF and glucose consumption but appreciately lower y gen consumption, indicating continued non-oxidative use of glucose in the hyperglycemic group. In general, regional CBF values exceeded the control value by 100–200% 3 min after ischemia and were reduced to 50% of control at 1 h after ischemia, at which time the rats were still comatose. In the brain stem of hyperglycemic rats, blood flow however remained elevated after ischemia. Thus, the significantly increased mortality observed in rats hyperglycemic before, during and after ischemia (Siemkowicz & Hansen 1978) was the result, not of impaired postischemic CBF but of ischemic or postischemic damage to brain cells. W suggest that the damaging factor in the hyperglycemic group is increased lactacidosis associated with prolonged anaerobic glycolysis.

Key words: Cerebral ischemia, cerebral blood flow hyperglycemia, postischemic recovery

..owicz & Hansen (1978) reported the finding high blood glucose in experimental animals ..cted to short episodes of global cerebral is..ia is associated with considerably reduced res..ion when compared to animals with normal or blood sugar. Myers & Yamaguchi (1976) as..ted the same observation with the greater lac..sis of brain tissue encountered in and after ..mia in monkeys with high blood sugar.

..hemic damage to the brain may occur during ..er the ischemic insult and may first affect the ..ion of cells (the "cellular factor") (Levy et al. Levy et al. 1975b) or the patency of vessels reflow) (Ames et al. 1968). Although recent ..es hav failed to detect gross impairments of ..tion after short episodes of brain ischemia in ..als with normal blood sugar (Ginsberg et al. ..; Levy et al. 1975b), it was possible that perfu.. impairments intervened in the immediate post ischemic period of hyperglycemic animals. There fore the present study was intended to reveal post-ischemic differences of cerebral blood flow or metabolic rates for oxygen and glucose between groups of rats with low high or normal blood glucose before a well-defined episode of complete cerebral ischemia lasting 10 min

MATERIALS AND METHODS

The brains of normo- hyper or hypoglycemic rats were rendered ischemic for 10 min. Gross regional cerebral blood flow and blood-brain glucose transfer rates, as well as whole-brain metabolic rates for oxygen and glucose, were determined by the method of Gjedde et al (1980)

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[1] Presented in part at the 101st meeting of the American Neurological Association, Washington, D.C. September 1978 and at the Symposium on Cerebral Metabolism and Neural Function. Bethesda, Md, May 1979

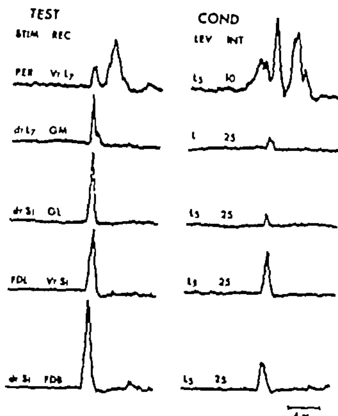


Fig 1 Facilitation and inhibition of hind limb reflexes by conditioning volleys in trunk skin nerves Left column unconditioned test reflexes elicited (STIM) and recorded (REC) as indicated Dr dorsal root Vr ventral root Right column test reflexes after conditioning volleys in skin nerves at different lumbar levels (LEV). Conditioning test intervals (INT) in ms See text

ditioned monosynaptic response represents a reflex response elicited by the conditioning stimulus itself as evidenced by other experiments These reflex responses in PER indicate the presence of excitatory connections between the dorsal trunk skin and motoneurons supplying ankle flexors and/or toe extensors Reflex discharges caused by the conditioning volley alone were never observed in any other limb nerve tested Inhibitory as well as facilitatory effects were elicited at stimulus strengths evoking reflex activity in the back muscles Weaker stimulation was ineffective and higher intensity did not change the general nature of these effects Conditioning stimulation delivered to dorsal skin nerves at other segmental levels (L_1–L_4) and to corresponding contralateral nerves had similar effects on the different test reflexes as described above

The reflex effects of stimulation applied to ventral skin nerves proved to be less marked than those originating from the dorsal skin nerves and displayed a higher degree of variability from one preparation to another A common pattern found was a slight facilitation of the test reflexes at conditioning intervals between 20 and 40 ms ... several occasions no signs of facilitation were observed Thus, following ventral skin nerve stimulation no distinct difference in reflex effects on motoneurons supplying flexors and extensors in the lower limb and in the foot ... established (cf dorsal skin nerve st... above)

The results from the test-conditioning experiments suggest that there are special ... for facilitation and inhibition of limb motor... from trunk skin The latencies at which the effects occur suggest reflex interaction through polysynaptic chains of interneurons. There ... evidence for the presence of reflex con... over several segmental levels ipsilaterally and contralaterally Some degree of reciprocity ... dorsal and ventral trunk skin seems to be ... but a prominent feature of trunk skin stim... inhibition of the ankle extensors in both ... Functionally in a standing position, the ... may promote the lowering of the body toward ground in order to avoid a stimulation applied ... dorsal side of the trunk In this respect the effects described can be regarded as part of ... drawal mechanisms

The results may have some bearing on findings in man of suppression of leg reflexes in painful states in the lumbar back region. In disc prolapses a depressed ankle or patellar ... may be due to blocking of nerve fibers ... roots In cases where no herniation is pre... depression of reflexes nevertheless ... mechanism is not plausible Instead some ... functional inhibition from pain afferents ... motoneuronpool has been suggested (Mooney & Robertson 1976) Our present results give ... mental evidence for the existence of ... mechanism in the cat

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Ph siological ariable of rats included i the pre nt study

e xpressed as mean ± S.E. Numbers in brackets indicate animals in which only arteriovenous deficits were

	Time after ischemia	Group Normal glucose	High glucose	Low glucose
of animals	Control	5	4	4
	3 min	6 (3)	6 (3)	5 (3)
	60 min	6	8	7
mHg)	Control	130 15	145 20	142_20
	3 min	142±10	148±7	128 5
	60 min	142+11	157±6	155±5
mHg)	Control	33 2	37±0.4	35±2
	3 min	37±2	33 2	35 2
	60 min	32±.	35±	32±0.3
	Control	7.47±0.02	7.41±0.03	7.39±0.02
	3 min	7.36+0.04	7.31+0.04	7.28±0.02
	60 min	7.45±0.05	7.32±0.02	7.37±0.03
(mmHg)	Control	100±3	102 13	101_8
	3 min	124±7	158±16	146 L
	60 min	109±5	111±7	93±9

ued 3 min after the termination of ischemia all ary manipulations could not be carried out in any t Consequently in these expts. measurements of ficits ere made in one group of animals and the measurements (flow etc) in another No variables rats in these two groups differed significantly with exception of the arterial plasma glucose concentration as higher in the groups in hich the superior al sinus as cannulated after ischemia (cf Table 2) glucose consumption (CMR_{glu}) was calculated from se a- deficit of one group and the cerebral blood of another hile the "undirectional" glucose flux as calculated from values observed in the same rat, alculated values for CMR_{glu} and J^* are not com-

n the animals are in respiratory steady-state x of 5 µC [^{14}C]butanol and 20 µCi [^{3}H]glucose in l Ringer HEPES buffer solution (Gjedde & Crone as rapidly injected into the femoral vein. Prior to g. the [H] glucose solution as air-dried and resus- d to eliminate olatile H-activity

rgrated concentration time curves of arterial blood produced mechanically as described previously de et al. 1980). Briefly one or tw arterial samples drawn into calibrated glass syringes at known and ant rate during and for 20 after injection At the end a s. ithdrawal was discontinued and the animal de ated ith rodent guillotine The brain as rapidly ed and divided bilaterally into the following regions, ighing from 30 to 100 mg each cerebellar hemisphere, ellar ermis, medulla and pons, midbrain, basal , m. parietal cortex and frontal, occipital, and tem- lobes The remainder of the brain as counted as

pe blood and tissue samples were rapidly transferred

to preweighed counting ials containing 1.5 ml of Sol- uene (Packard) and isopropanol mixture (1 1). The vials and samples were then reweighed, blanched with 0.5 ml 35% hydrogen peroxide and stored overnight at 50°C. The next morning, preparation was completed by addition of 20 ml of an Instagel (Packard) and 0.5 n HCl mixture (9: 1) The vials ere allowed to stabilize at counting temper ature for 24 h prior to counting in Packard Tri-Carb 425 liquid scintillation spectrometer

Preparations of n-[1 ^{14}C]butanol, D-[1 H]glucose and [^{3}H]dextran ere obtained from New England Nuclear Corporation with specific activities of 1.86 mCi mmol^{-1} 18 Ci mmol^{-1} and 318 mCi g^{-1} respectively

RESULTS

Arterial tensions of oxygen and carbon dioxide arterial pH and mean arterial blood pressure were monitored in all rats and met the following criteria established for inclusion in the present study: Pa_{O_2} >100 mmHg, 30<Pa_{CO_2} <40 mmHg and MABP>100 mmHg before and 3 min after the end of ischemia (Table 1). Arterial concentrations of glucose and oxygen varied as shown in Table 2.

Cerebral blood flow d ring hemia

Gross regional blood flow rates were measured in two rats 5 min after the onset of ischemia (Table 3) The flow rates were uniformly below 1% of the normal rate

consisting of an i v bolus administration of a mixture of labeled butanol and labeled D-glucose with subsequent determination of the brain content of these tracers. Whole brain metabolic rates for glucose and O were calculated from the product of cerebral arterio-venous deficits and whole-brain blood flow rates

Ischemic model

The production of 10 min of cerebral ischemia in the rat has been described in detail elsewhere (Siemkowicz & Hansen 1978) However absolute values of blood flow during ischemia were not assessed previously

Following ether induction the rats were intubated with steel tubes Three min prior to ischemia all animal received 50 IU kg heparin sodium i.a and halothane administration was discontinued Between 1 min before and 1 min after the onset of ischemia arterial blood was slowly removed until the mean arterial blood pressure reached 50 mmHg during ischemia Cerebral ischemia was introduced by inflation of a pneumatic cuff around the neck to a pressure of one atmosphere

After 10 min of ischemia the cuff was rapidly released and the arterial blood reinfused in 30–45 s To the la t ml of blood 5 μg adrenaline was added causing the mean arterial blood pressure to rise to 150–180 mmHg All rat treated with insulin received an i p injection of 50% glucose solution at the end of ischemia in order to raise plasma glucose to levels comparable with those of the control group in the postischemic period

Determination of cerebral blood flow and blood-brain glucose transfer rat

Whole brain and regional cerebral blood flow rates were calculated from tissue and arterial blood sample contents of labeled butanol 20 s (= T) after an i v injection using an equation relating cerebral blood flow per unit weight of brain (f^{bl}) the amount of butanol present in unit weight of brain ($C_{br}(T)$) and the amount of butanol present in an arterial sample ($Q_a(T)$) collected at a constant and known rate (F^{bl})

$$f^{bl} = \frac{1}{E(T)} F^{bl} \frac{C_{br}(T)}{Q_a(T)} \qquad (1)$$

where E(T) i the net extraction fraction in the time T

Whole brain and regional unidirectional blood-brain glucose transfer rates were calculated from extravascular brain ti sue and arterial blood sample contents of labeled glucose assuming no los of labeled glucose from the brain during the experiment

$$J = C_a^{pl} F_a^{pl} \frac{C_{ev}(T)}{Q_a^*(T)} \qquad (2)$$

where J represents the unidirectional glucose transfer rate per unit weight of brain C_a^{pl} the arterial plasma glucose concentration F_a^{pl} the plasma flow into the sampling syringe $C_{ev}(T)$ the extravascular content of labeled glucose in unit weight of brain and $Q_a(T)$ the content of labeled glucose in the arterial sample

Whole brain values were obtained by adding from regional samples and left-over tissue [illegible] fraction of extracted tracer glucose was calculated [illegible] the equation

$$E_{glc} = \frac{J}{C_a^{pl}\ f^{pl}}$$

E(T) for butanol was estimated from the rate of [illegible] butanol from the brain and the rate of decay of the [illegible] concentration of labeled butanol Since E(T) [illegible] ly with the cerebral blood flow as explained [illegible] previously (Gjedde et al. 1980), equation (1) [illegible] solved by an iterative procedure

The rate of decay of the monoexponential [illegible] centration curve for butanol, as well as the [illegible] labeled glucose placed intravascularly in brain at the [illegible] of decapitation were calculated from the [illegible] labeled butanol and glucose in arterial blood at the [illegible] The volume of pla ma in brain was determined [illegible] labeled dextran in separate expts. In these [illegible] were injected with [H] dextran (molar weight [illegible] mol) and the H-activity per unit weight of brain [illegible] volume of arterial plasma determined at the [illegible] capitation after 3 min circulation.

General proced re

Male Wistar rat were divided in 3 groups [illegible] *normal gluco e* group received no pretreatment [illegible] the *low gluc e* group received IU kg [illegible] (Leo) i p 1 h prior to study Rats in the *high* [illegible] received 17 mmol kg D-glucose i p 1 h prior [illegible]

The 300–350 g rats were kept specific-pathogen [illegible] were starved overnight Anesthesia [illegible] ether The animals were paralyzed with 3 [illegible] suxamethonium and were intubated and [illegible] 1% halothane vaporized in a mixture of [illegible] oxygen (70 30)

Catheters were placed in the tail artery [illegible] arteries one femoral vein, and in most rats [illegible] below the superior sagittal sinus of the brain [illegible] of the sinus was performed as described [illegible] (Gjedde et al 1980)

Arterial and cerebral venous blood samples [illegible] sayed for pH P and P_{O_2} with [illegible] (Radiometer) Arterial hematocrit was measured [illegible] trifugation Plasma concentrations of glucose [illegible] ured in freshly sampled arterial and cerebral venous [illegible] ma by the glucose oxidase method (Christensen [illegible] Hjelm & De Verdier 1963) Arterial and [illegible] concentrations of oxygen were determined after [illegible] monoxide displacement in a galvanic fuel cell [illegible] Co Lexington Instruments) The animals were [illegible] ered to be in respiratory steady-state when Pa_{CO_2} [illegible] ples taken at least 10 min apart agreed within 10% [illegible] al blood pres ure was continuously recorded [illegible] gauge transducer

Since metabolic rates were calculated by multiplic[illegible] of arteriovenou deficits with blood (oxygen) or [illegible] (glucose) flows in brain care was taken to draw the [illegible] ples for determination of arteriovenous deficits as [illegible] before flow measurements as possible In the

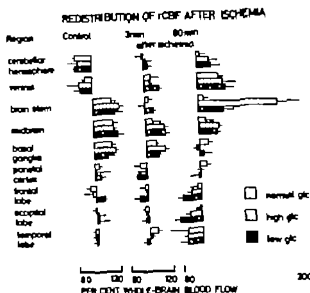


Fig. 1. Regional distribution of cerebral blood flow rates in rats with different plasma glucose levels at control condition and 3 and 60 min after cerebral ischemia of 10 min duration. Each bar represents per cent of whole-brain blood flow (±S.E.) at respective conditions.

...ol state while at 60 min, the cerebral blood ... was higher in infratentorial regions than in the ...atentorial regions. However no significant dif...ces in distribution were observed between the ...oups of rats with the exception of the brain stem of rats pretreated with glucose in which an increase to 175% of the whole-brain average was seen 60 min after ischemia, indicating no post-ischemic reduction of absolute blood flow i[n] this region.

The selective reduction of the blood flow of sup-

...e 4. *Whole-brain blood flow, metabolic rat[e]s for [o]xyge[n] and glucose, and the oxygen-glucose ind[e]x ...re ...nd after 10 m[in] of complet[e] cerebral [is]chemia*

...es are expressed as mean ± S.E.

...able	Time after ischemia	Group: Normal glucose	High glucose	Low glucose
... (ml (100g) ... min ...)	Control	129±8	87±20	116±13
	3 min	168 28	192±38	299±52*
	60 min	65±6*	64±11	46±4*
...O_2 (µmol (100 g) ... min ...)	Control	379±49	433±76	313±63
	3 min	179±58*	205±71*	321±116
	60 min	196±10*	94±9*	174±22
R_{glc} (µmol (100 g) ... min⁻¹)	Control	67±10	74±8	33±6*
	3 min	111±35	118±40	187±64
	60 min	41±12*	38±11	27±7*
...gen, glucose index (%)	Control	95	97	158
	3 min	27	28	28
	60 min	80	42	107

...significantly different from pre-ischemic value at $P<0.05$ or less.
...significantly different from value at 3 min postischemia at $P<0.05$ or less.
...significantly different from mean value of the other groups at $P<0.05$ or less.

Table 2 *Arterial concentration of glucose (plasma) and oxygen (blood) before and after ischemia*
Values are expressed as mean ± S E Numbers in brackets indicate animals in which only arteriovenous ... measured

Variable	Time after ischemia	Group Normal glucose	High glucose	Low glucose
glc (mM)	Control	10 4±0 9	30 8±0 7	1.8+0 6
	3 min	11 1±1 1 (14 1±1)	36.3± .5 (35.5±..4)	7.3+1.6 (13 9±1.3)
	60 min	9 9±1 4	33 0±5 0	11 7±..0
O (mM)	Control	9 1±0 1	10 4±0..	9 1±0.7
	3 min	9 4±0.3	10.3±0 1	9 7+0 3
	60 min	9.8±0.3	10 0±0.5	10.4±0.3

Cerebral blood flow and metabolic rate before ischemia

The average whole brain blood flow rates and the metabolic rates for oxygen and glucose of the three groups before ischemia are shown in Table 4 Glucose consumption was insignificantly increased in the *high glucose* group and markedly reduced in the *low glucose* group

Oxygen consumption was similar in the three groups The oxygen glucose index (OGI) was near 100% (OGI = CMR$_O$ 100/CMR$_{glc}$ 6) in the *normal* and *high glucose* groups before ischemia In the *low glucose* group on the other hand oxygen consumption exceeded the rate calculated stoichiometrically from glucose consumption indicating metabolic use of othe substrates than glucose as also noted by Norberg & Siesjö (1976)

Table 3 *Cerebral blood flow during ischemia*

Region	CBF (ml (100 g) min)
Cerebellar hemisphere	0 2
Vermis	0 1
Pons	0 7
Midbrain	0 4
Basal ganglia	0..
Parietal cortex	0 1
Frontal lobe	0 05
Occipital lobe	0.03
Temporal lobe	0.1
Cervical cord	11 4
Pa$_O$ (mmHg)	118
Pa (mmHg)	33
pH	7.51
MABP (mmHg)	50

Mean of estimates at 5 min of ischemia.

Cerebral blood flow and metabolic rate after ischemia

Three min after the end of the ischemia ... whole brain blood flow was increased in ... groups of rats No significant differences were ... served between the groups (Table 4). The ... rate for oxygen in the normal and high ... groups was decreased to about 50% of ... unaltered in the low glucose group. The ... rate for glucose was slightly increased in the ... and *high glucose* groups and greatly ... the *low glucose* group in which blood glucose ... been raised to normoglycemic levels just ... end of the ischemic episode. The OGI ... proximately 30% in all 3 groups indicating ... only one third of the glucose used by ... oxidized

Sixty min after ischemia whole-brain ... was about 50% of the control value in all 3 ... The oxygen consumption was lower than ... all rats but significantly lower in the high ... group than in the other groups (Table ... metabolic rate for glucose was reduced ... groups

Sixty min after ischemia the OGI was near ... in the *normal* and *low glucose* groups. In the ... *glucose* group however the animals continued ... use glucose non-oxidatively Thus the high ... group could be identified as the group with ... lowest rate of oxygen consumption and ... non-oxidative use of glucose (about 60%) ... after ischemia.

The regional distribution of cerebral blood ... rates is shown in Fig 1 Three minutes after ... chemia the dis... was essentially as ...

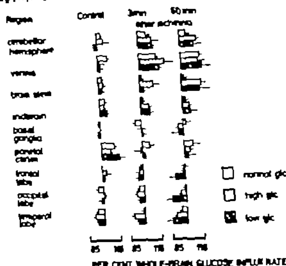


Fig. 2. Regional distribution of unidirectional blood-brain glucose flow in rats with different plasma glucose levels in control condition and 3 and 60 min after cerebral ischemia of 10 min duration. Each bar represents per cent of whole-brain glucose influx rate (+ S.E.) at respective conditions.

...s marked unevenness of the distribution of the ...unlikely. Third, the favorable CBF : CMR_{O_2} ... speak against any flow-induced oxygen short... In the high glucose group at 1 h after ischemia, ... ratio was even higher than normal (0.7).

...urs, the high mortality following ischemia in ...rglycemic rats was not caused by insufficiency ...lood flow. It must be related to the delayed ...bolic depression (fall in CMR_{O_2}) found in all ...ups after ischemia and observed to be particular... ...rominent in the group of highest mortality, i.e. *hyperglycemic rats.*

...he metabolic depression in the high glucose ...up at 1 h after ischemia may represent inhibition ...xidative enzymes caused by the severe tissue ...acidosis. Low metabolic rate shortly after is...mia has been observed by several investigators ...nsberg et al. 1978, Snyder et al. 1975, Steen et al. ...(8). Its presence, however, is not a simple con...uence of reduced functional state (e.g. trans...sion failure) of the brain after ischemia (postis...mic coma) since the rats of the three groups ...re equally unresponsive immediately after is...mia. The delayed metabolic depression of the ...gh glucose group after ischemia strongly suggest ...at the metabolism may be influenced by factors peculiar to hyperglycemic ischemia, e.g. lactacidosis.

What is known of the metabolic events after hyperglycemic ischemia that may point to a cause of the insufficient oxidation of glucose. The fundamental metabolic events of the immediate postischemic period appear to mirror those of ischemia itself. First, restoration of blood flow and oxygen delivery triggers the resumption of mitochondrial function. Inhibition of phosphofructokinase activity by increased hydrogen ion concentration (Siesjö 1978) ensures that lactate is reconverted to pyruvate before glycolysis is resumed. Labile phosphates accumulate and the (Na⁺-K⁺) ATPase proceeds to normalize extracellular ion concentrations and volume.

The duration of these events after short-lasting ischemia is known. Lactate begins to fall and labile phosphates reach preischemic levels within 2 min (Drewes & Gilboe 1973, Ljunggren et al. 1974) and the extracellular ion concentrations and volume return to normal in 3–4 min (Siemkowicz & Hansen 1979). Lactate continues to fall for a period of time determined by its end-ischemic concentration (Ljunggren et al. 1974).

From studies of the accumulation of labile phos-

Table 5 *Cerebral plasma volume and unidirectional blood brain transfer of glucose*
Values are expressed as mean ± S E

Variable	Time after ischemia	Group: Normal glucose	High glucose	Low glucose
Whole-brain plasma volume (ml (100 g))	Control	0 88±0 08	0.82±0 18	[illegible]
	3 min	1 _5±0 35	1 06_0 _	1 07 0 15
	60 min	0 93±0 17	0 80±0 17	0 83±0 19
Unidirectional blood-brain glucose transfer (µmol/100 g min)	Control	137±10	174±19	[illegible]
	3 min	70_±30	195±38	157 13
	60 min	86±8	149±29	[illegible]
Plasma extraction fraction (ratio)	Control	0 19±0 01	0 15±0 03	0 37±[illegible]
	3 min	0 18±0 0_	0 09±0 01	0 16±[illegible]
	60 min	0 30±0 0_	0 17±0 0_	[illegible]
Transfer constant (k) (ml g min)	Control	0 13	0 056	0 _5
	3 min	0 143	0 074	0_16
	60 min	0 087	0 045	0 079

ratentorial regions in favor of infratentorial regions 60 min after ischemia resembles the response seen in metabolic coma

Blood brain glucose transfer

The unidirectional transport of glucose from blood to brain and the extraction fraction of tracer glucose are recorded in Table 5 Regional unidirectional blood-brain glucose transfer rates are summarized in Fig 7

Because glucose is transported from blood to brain by a saturable carrier mechanism it was of interest to calculate the change of blood brain glucose transport after ischemia in the 3 groups of rats

In all rats the change of glucose transfer was positively correlated to the change of cerebral blood flow (Tables 4 and 5)

The unidirectional glucose transport rate has been expressed by Sokoloff et al (1977) as a transfer constant k multiplied by the concentration of glucose in arterial plasma The values of k calculated by dividing the unidirectional glucose flux by the arterial plasma concentration are shown in Table 5 It is evident that k the constancy of which forms the basis of the calculation of cerebral glucose consumption with 2-deoxy D-glucose (Sokoloff et al 1977) varies markedly

DISCUSSION

We investigated the mechanisms underlying [illegible] servation made previously by Siemkowicz [illegible] sen (1978) that hyperglycemia reduces the [illegible] of rats after 10 min of complete cerebral [illegible]

No differences of whole-brain or regional [illegible] brain stem) blood flow distinguished the [illegible] jected to hyperglycemic ischemia from [illegible] jected to normo- or hypoglycemic ischemia [illegible] though in other respects differences were [illegible] in the course of the study these differences [illegible] veloped in the first hour after ischemia and were [illegible] present at 3 min The differences included [illegible] cerebral oxygen consumption greater [illegible] tive use of glucose and greater brain stem [illegible] flow

It was our intention to relate CBF to the [illegible] metabolic demands after ischemia Although we [illegible] not measure blood flow in regions weighing [illegible] than 30–100 mg the following observations [illegible] the assumption that insufficient postischemic [illegible] flow played no role in the development of [illegible] chemic encephalopathy

First the hyperglycemic rats had a higher [illegible] blood flow relative to the preischemic value [illegible] the other groups at 1 h after ischemia Second [illegible] high unidirectional blood glucose transfer rate [illegible] ble 5) compared to [illegible] hyperglycemic [illegible]

ometrial cholinergic secretory responses during ous cycle pregnancy and after estrogen and/or jesterone treatment of the guinea-pig

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HAMMARSTRÖM M Endometrial cholinergic secretory responses during estrous cycle pregnancy and after estrogen and/or progesterone treatment of the guinea-pig. Acta Physiol Scand 1980, 110: 233–240. Received 27 Nov 1979 ISSN 0001-6772. Department of Physiology Karolinska Institutet, Stockholm, Sweden.

During estrous cycle as well as during pregnancy and after treatment with estrogen and/or progesterone uterine mucus secretory responses were studied as amount of carbohydrate released from everted guinea-pig uteri after transmural nerve stimulation or carbachol administration. In estrous cycle both stimuli increased secretion by about 20% except for the late diestrus phase, when no secretory responses were seen. In pregnancy secretory response was noted at implantation time From mid pregnancy and onwards secretory responses of increasing magnitude were obtained. Secretory responses persisted post partum. Evidence of an adrenergic inhibitory influence on the neurogenic cholinergic responses during metestrus was noted Both stimuli increased carbohydrate release by about 50% above resting level in uteri from animals receiving combined hormone treatment. In uteri of animals receiving estrogen only the increase was about 20% No definite secretory response was obtained in uteri from animals receiving progesterone only It is suggested that both hormones are necessary for full cholinergic secretory response to nerve stimulation or muscarinic drugs. It is concluded that muscarinic stimulation of the endometrium results in mucus secretion during all crucial events during the reproductive cycle. Furthermore a cholinergic secretory innervation seems to be present at all these times.

Key word Uterus endometrium, cholinergic nerves, mucus secretion, estrous cycle, pregnancy estrogen, progesterone

previous report (Hammarström & Sjöstrand) evidence of a cholinergic, presumably sym-hic secretomotor innervation of the guinea-pig metrium was presented. The work presented ıt paper as based on experiments on uteri ght into artifical secretory phase by a com-ion of estrogen and progesterone treatment. ıne secretion was calculated from amounts of ohydrate released from everted uteri placed in n baths.

ndometrial function is dependent on female sex nones (vide e.g. Hitschmann & Adler 1908, es, Hertig & Rock 1950, Themann & Schünke Dallenbach-Hellweg 1969 Laws 1973). Ex ous female sex hormones as w ll as pregnancy : dramatic and diverse effect on myometrial rvation (cf Sjöberg 1967 Owman, Sjöberg & Sjöstrand 1974 Thorbert 1978) as well as on the myometrium (e.g. Marshall 1970).

Thus a natural follow-up seemed to be to investigate secretory responses to field stimulation of intramural nerves and to a muscarinic drug i.e. carbachol during estrous cycle of virgin female guinea-pigs and during pregnancy as well as post partum, i.e. when ovulation and repeated fertilization ordinarily occurs in the guinea-pig. It also appeared to be of interest to investigate secretory responses of uteri dominated by excess of estrogen or progesterone

MATERIAL AND METHODS

Animals. Mottled or albino virgin guinea-pigs (400–500 g) were used For the study on the reproductive cycle the

phates (Ljunggren et al 1974) and the restitution of extracellular ion concentrations (Siemkowicz & Hansen 1979) it is known that these metabolic events proceed at the same rate after hyper and normoglycemic ischemia Therefore the only characteristic which distinguishes the hyperglycemic ischemic recovery from normo- and hypoglycemic recovery is a lingering lactacidosis

Our results indicate that circulatory insufficiency after ischemia does not distinguish the high glucose group from the other groups and that the deleterious effect of glucose pretreatment on the revival of rats after cerebral ischemia may involve abnormalities of oxidative metabolism This conclusion is supported by the failure of Welsh et al (1978) to find any correlation between regional cerebral blood flow and deranged metabolite concentrations after ischemia in hyperglycemic animals

This study was supported by grants from the Danish Medical Research Council (no 51 -8173) and Lönborg Madsen s Foundation The authors wish to thank Ellen Munch and Susanne Korsholm for expert technical assistance and Berit Ree and Hildegard Mosthaf for careful typing of the manuscript

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Weight endom trial carbohyd ate concentration nd resting ecretion level of teri from gui ea-
ted ith estrogen and/or progesterone
S.D number of experiments within brackets Concerning uterine wet eight, there were statistically significant
es between the groups. N such differences were seen with respect to the endometrial carbohydrate concentra-
e resting secretion level of combined treatment group was lower ($P<0.01$) than that of the two other groups

	Uterine wet weight (g)	Endometrial carbo-hydrate concentration (µmol/g)	Resting secretion level (µmol/10 ml)
n and progesterone treatment	0.73±0.25 (25)	44 16 (19)	0.47±0.17 (21)
treatment	0.93±0.40 (30)	39±14 (21)	0.77+0.35 (20)
erone treatment	0.46±0.14 (14)	42±26 (10)	0.60_0.23 (12)
ed treatment versus estrogen ent	$P<0.05$		($P<0.01$)
ed treatment versus progesterone ent	$P<0.001$	n.	n.
n treatment versus progesterone ent	$P<0.001$	n.	n.s

ster After shaking the homogenate was cen- 1 4.5 ml of the supernatant was removed and to it 18.5 10 M indole as added hereafter the sam- s heated and the carbohydrate content was deter- using an internal standard (vide Hammarstrom & nd 1979)

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LTS AND COMMENTS

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Similary during pregnancy mucosal carbohydrate concentration was rather uniform Maximum concentration was noticed in early pregnancy (day 16–18)

Resting arbohydrate secretion lev l
(Tables 1,2 and 3)

During the estrous cycle no statistically significant variation in resting secretion level was noted In *pregnancy* maximum secretion level was noted at day 16–18, i.e when mucosal carbohydrate concentration was largest (see above) A second maximum was noted at day 63–68 of pregnancy No significant difference in resting secretion level between the groups receiving single hormone treatment was noted. However the group receiving combined treatment had lower resting secretion level

Field stimulation of nerv
(Figs. 1 and 2)

Estrow cycle (Fig 1 A.) A secretory response to field stimulation was elicited at the days around ovulation, i.e estrus (day 16–) (+20 %) On day 3–5 (metestrus) field stimulation caused an inhibition of carbohydrate secretion (−15 %) The discrepancy in effect on carbohydrate secretion caused by field stimulation and carbachol (see below) respectively together with previous results that possibly

Table 1 *Endometrial carbohydrate concentration and resting secretion level during the estrous cycle*

There was no statistically significant difference between the groups Mean ± S D number of experiments within bracket

Day in estrous cycle	Endometrial carbohydrate concentration (µmol/g)	Resting secretion level (µmol/10 min)
16–	50±18 (18)	0 74±0 4 (1)
3–5	43±16 (25)	0 71±0 25 (18)
6–7	49±16 (14)	0 61±0 36 (13)
8–11	51±17 (13)	0 85±0 7 (14)
1 15	43±8 (1)	0 75±0 36 (10)

animals were controlled daily concerning the status of the vaginal closure membrane Only animals showing regular cycles were used

Animals used for the study of hormone effects were oophorectomized under sodium pentobarbital anesthesia (30–40 mg/kg) (when necessary supplemented with ether)

Determination of ovulation in the estrous cycle When two normal cycles had been observed a smear was taken when the membrane was open The day of maximum cornification before leucocytic influx was designated day 1 (Stockard & Papanicolaou 1917 1919 Bland & Donovan 1970 Thorbert 1978)

Determination of conception day in pregnancy 4–5 females were caged with one male Day 1 of pregnancy was determined as the second day in the last period of open vaginal membrane (Kelly & Papanicolaou 1927 Phoenix 1970) Gestational length was controlled by palpation early in pregnancy

Experimental groups and uteri taken in the estrous cycle at day 16–2 3–5 6–7 8–11 and 1 15 Experiments on pregnant animals were undertaken at day 6–8 16–18 28–3 38–4 48–5 63–68 and 4–1 h post partum In animals used for post partum experiments labour was sometimes induced with oxytocin (2 IE i m occasionally repeated once (Phoenix 1970))

Dissecting and mounting procedure The animals were stunned and bled In pregnant animals the fetuses, placentae and membranes were removed The organs were then mounted according to Hammarström & Sjöstrand (1979) Horns from animals taken from experiments at pregnancy day 40 or later were mounted in an organ bath of 100 ml volume

Hormone treatment Three weeks after castration treatment with either estradiol valerate (Progynon) 6 mg/kg or hydroxyprogesterone caproate (Proluton®) 150 mg/kg or both hormones every second day was started and continued for 10–14 days

Experimental performance Experiments were performed following the schedule given by Hammarström & Sjöstrand (1979)

After 80 min accomodation in ordinary Tyrode solution (36°C) changed every 20 min the sampling was started Five sampling periods, lasting for 10 min in Tyrode solution where glucose was replaced by H_2O formed The periods were interrupted by rest of 20 min in ordinary Tyrode solution First the sampling period was used for determination of drate resting secretion level The second was used for studying the effect of continuous stimulation or administration of drugs or hor experiments only three sampling periods In these cases there were two resting periods one stimulation period

Estimation of uterine carbohydrate formed as described in detail by Hammarström strand (1979) The secretory response was calc percent increase in carbohydrate secretion the secretion level using the mean of the resting levels as matching value Concerning the large (from late pregnancy experiments) the following tions were introduced The samples were evap thermostatic bath (40°C) The dry residue was double the amount of water and 14 4 M H_2SO_4 and 9 ml respectively) After centrifugation supernatant was removed and 0.15 ml 5×10 was added The values of secretion level were for the dilution Recovery of added glucose 10 1×10⁻⁴ ×10 M) yielded 67+6 (M S Recovery corrected for the dilution yielded (M±S D n=8)

Determination of endometrial carbohydrate After the experiment the endometrial layer was from the myometrium and a small piece was analysis Each piece was homogenized by quartz sand in a mortar containing 0 5 ml of 14 Rinsing was performed with 4 ml 14 4 M H_2SO_4

Table 2 *Endometrial carbohydrate concentration and resting secretion level during pregnancy*

Mean ± S D number of experiments within br

Day of pregnancy	Endometrial carbohydrate concentration (µmol/g)	Resting secretion level (µmol/10 m
6–8	4 ±11 (14)	0 71+0
16–18	53±11 (7)	1.6 ±0 1
28–3	35±1 (9)	0.71±0
38–4	33±4 (8)	0 60±0 4
48–5	35±15 (13)	0.95±0 4
63–68	38±16 (17)	1 40±0
4 h p p	30±12 (17)	0 50±0

This value is higher ($P<0.01$) than values at da and 4 h p.p

This value is higher ($P<0.001$–0.05) than all values in the group

The level is higher ($P<0.001$) than the levels of groups except the day 63–68 group

The level is higher ($P<0.01$) than the levels of groups except for day 16–18 group and day

This value is lower ($P<0.01$) than that of da group

Weight ndometrial arbohydrate concentration and r sting ecretion level of ut ri f un guinea ed th estrogen and/ or progesterone

S D number of experiments within bracket Concerning uterine wet eight there were tat tically signif ant es between the groups N such differences were seen with respect to the endometrial carbohydrate concentra- e resting secretion level of combined treatment group was lower (P<0 01) than that of the tw other groups

	Uterine wet weight (g)	Endometrial carbo-hydrate concentration (µmol g)	Resting secretion level (µmol 10 min)
and progesterone treatment	0 73 0 .5 (25)	44 16 (19)	0 47 0 17 (21)
treatment	0 93 0 40 (30)	39 14 (21)	0 ~ 0 35 (70)
rone treatment	0 46 ±0 14 (14)	42 26 (10)	0 46 0 23 (1 1
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Hormone treatm nt Three week after castration treatment with either estradiol valerate (Progynon) 6 mg/kg or hydroxyprogesterone caproate (Proluton) 150 mg/kg or both hormone every second day was started and continued for 10–14 days

Experimental performance Experiments were performed following the schedule given by Hammarström & Sjöstrand (1979)

After 80 min accomodation in ordinary Tyrode solution (36°C) changed every 20 ml the sampling w started Five sampling period lasting for 10 min in Tyrode solution where glucose was replaced by NaCl formed The periods were interrupted by [illegible] of 20 min in ord nary Tyrode solution First [illegible] sampling period was used for determination [illegible] drate resting secretion level The second [illegible] was used for studying the effect of [illegible] stimulation or administration of drugs [illegible] experiments only three sampling periods were [illegible] In these cases there were two resting periods [illegible] one stimulation period

Estimation of [illegible] carbohydrate [illegible] formed as described in detail by Hammarstrand (1979) The secretory response was [illegible] percent increase in carbohydrate secretion [illegible] secretion level using the mean of the resting levels as matching value Concerning the [illegible] (from late pregnancy experiments) the following [illegible] tions were introduced The samples were [illegible] thermostatic bath (40°C). The dry residue [illegible] double the amount of water and [illegible] and 9 ml respectively) After centrifugation [illegible] supernatant was removed and 0.15 ml [illegible] was added The values of secretion level were [illegible] for the dilution Recovery of added glucose [illegible] 10 [illegible] 1×10 [illegible] ×10⁻⁴ M)) yielded 67+6 [illegible] Recovery corrected for the dilution yielded [illegible] (M±S D n=8)

Determination of endometrial carbohydrate After the experiment the endometrial layer was [illegible] from the myometrium and a small piece was [illegible] analysis Each piece was homogenized by [illegible] quarts sand in a mortar containing 0.5 ml of [illegible] Rinsing wa performed with 4 ml [illegible]

Table 2 *Endometrial carbohydrate conc[illegible] and resting secretion level during pregnan[illegible]*

Mean ± S D number of experiments within [illegible]

Days of pregnancy	Endometrial carbohydrate concentration (μmol/g)	Resting secretion level (μmol/10 m[illegible])
6–8	42±11 (14)	0 71±0 [illegible]
16–18	53±11 (7)	1 6_±0.1 [illegible]
28–32	35±1 (9)	0.73±0 [illegible]
38–4	33±4 (8)	0 60±0.2[illegible]
48–52	35±15 (13)	0.95+0 41 [illegible]
63–68	38±16 (17)	1 40±0 [illegible]
4 h p p	30±12 (17)	0 50±0 25 [illegible]

This value i higher ($P<0.01$) than values a[illegible] and 4 h p p

This value is higher ($P<0.001$–0.05) than all [illegible] ues in the group

The level is higher ($P<0.001$) than the levels of [illegible] groups except the day 63–68 group

The level is higher ($P<0.01$) than the levels [illegible] group xcept for day 16–18 group and day 48–52 [illegible]

This value is lower ($P<0.01$) than that of [illegible] group

...eight endometrial arbohydrate concentration and resting ecretion le el of uteri from guinea-... with estrogen and/or progesterone

D number of experiments within brackets. Concerning uterine wet weight, there were statistically significant ... between the groups. N such differences were seen with respect to the endometrial carbohydrate concentra... resting secretion level of combined treatment group was lower ($P<0.01$) than that of the tw other groups

	Uterine wet weight (g)	Endometrial carbohydrate concentration (μmol/g)	Resting secretion level (μmol/10 min)
...and progesterone treatment	0.73±0 .5 (23)	44±16 (19)	0.47 0.17 (21)
...treatment	0.93+0.40 (30)	39 14 (.1)	0.7°±0.35 (20)
...one treatment	0.46+0.14 (14)	42±26 (10)	0.60 0.23 (12)
...d treatment ersus estrogen ...nt	$P<0.05$	s.	($P<0.01$)
...d treatment versus progesterone ...nt	$P<0.001$	n.s.	s.
...treatment versus progesterone ...nt	$P<0.001$	n.s.	s.

...ater After shaking the homogenate was cen... 4.5 ml of the supernatant was removed and to it ... 8.5 10 M indole was added whereafter the sam... heated and the carbohydrate content was deter... ...using an internal standard (ide Hammarström & ...nd 1979)

...stimulation was applied at standard rate of 0.5 ... ms, 30 V which had been found to give optimal ...ses (vide Hammarström & Sjöstrand 1979) At ...ncy day 16–18, 28–32 38–42, the uterine all was ...split hen the placentae and fetuses were expelled. ...made it impossible to obtain an adequate spread of ...t through the tissues on transmural stimulation. ...fore field stimulation experiments ere omitted on ...days

Apart from the hormones obtained from Scher... G carbamylcholine chloride (Carbachol) (Sigma), ...lamine hydrobromide (Sigma), phentolamine ...de (Ciba), methoxamine hydrochloride (BDH), and ...cin (Ferrosan) were used

...ysis The Students -test or hen necessary the approximative -test (vide Remington & Schork ...as used for statistical comparison between the

...k solutions for composition see Hammarström & ...rand (1979)

...ULTS AND COMMENTS

...osal arboh drat oncentration
...les 1 2 and 3)

...roughout the estrous cycle and after hormone ...ment little variation in mucosal carbohydrate ...centration was seen The similarity in mucosal ...ohydrate concentration is probably explained ... he fact that female sex hormones alone or in ...bination change the relative distribution and type of polysaccharides in epithelial glandular or stromal tissues rather than affect total carbohydrate concentration as such (cf Schmidt Matthiesen 1963).

Similary during pregnancy mucosal carbohydrate concentration wa rather uniform. Maximum concentration was noticed in early pregnancy (day 16–18)

Rest g carbohydrate secretion level
(Tables 1 and 3)

During the estrous cycle no statistically significant variation in resting secretion level was noted In *pregnancy* maximum secretion level was noted at day 16–18 e when mucosal carbohydrate concentration was largest (see above) A second maximum was noted at day 63–68 of pregnancy No significant difference in resting secretion level between the groups receiving ingle hormone treatment was noted. However the group receiving combined treatment had lower resting secretion level

Field stimulation f nerve
(Figs 1 and)

Estrous cycle (Fig 1A.) A secretory response to field stimulation was elicited at the days around ovulation i e estrus (day 16–2) (+20%) On day 3–5 (metestrus) field stimulation caused an inhibition of carbohydrate secretion (−15%) The discrepancy in effect on carbohydrate secretion caused by field stimulation and carbachol (see below) respectively together with previous results that possibly

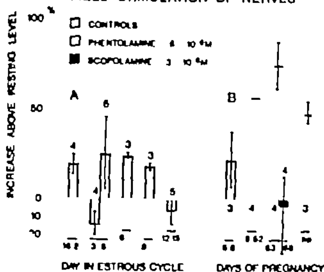


Fig. 1. Secretory responses (carbohydrate release) of guinea-pig uteri t transmural stimulation during estrous cycle (A) and during pregnancy (B). Uteri were stimulated continuously with 0.5 Hz, 0.5 ms, 30 V during 10 min periods. Responses in this and following figures are expressed as per cent of resting secretion level, i.e. carbohydrate release during control periods. For further explanation se Material and Method. (A) Responses are seen at all intervals except late diestrus (day 1 15). Note the reversed answer after phentolamine admini tration at day 3–5. (B) A response is seen at time of implantation (day 6–8). A gre ter response is seen in late pregnancy. The response persi ts post partum. Scopolamine abolishes the response at day 63–68 (mean and range number f experiment indicated by figures at column.)

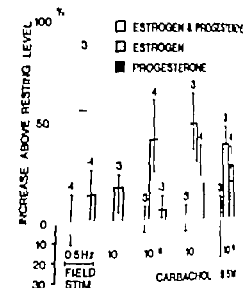


Fig. Effects of field stimulat n (0.5 Hz) and carbachol on carbohydrate secretion from payed guinea-pigs treated with excess of e progesterone. Responses are not seen in the prog treated group but in the estrogen treated group responses are seen in the group receiving both (cf. responses of pregnant uteri Fig. 1 and 4)

might suggest an inhibitory adrenergic influence on the uterine carbohydrate secretion (vide Figs. 1 and 3 in Hammarström & Sjöstrand 1979) prompted a test with the α blocker phentolamine. This drug unmasked a secretory response of about +25%, i.e. of the same magnitude as that elicited by carbachol. Secretory reponses were obtained from day 6 until day 11 (early diestrus) of the estrous cycle (+20%) without treatment with phentolamine. In late diestrus (day 1–15) (i.e. when serum levels of progesterone are low (Feder, Resko & Goy 1968, Croix & Franchimont 1975)) and when epithelium is atrophic, low and cuboidal (Stockard & Papanicolaou 1917, Burgos & Wislocki 1956, 1958) no secretory response could be noted.

Pregnancy (Fig. 1B). At time of implantation (day 6–8) a secretory response was elicited (+20%). From time of placentation (at about day 10) until day 40 field stimulation was impossible to accomplish (see Material and Methods). In late pregnancy the secretory responses were of greater magnitude and reached +57% at day 50 and day 65. 4–12 h post partum a response could be noted (+47%) (i.e. when the para regularly and refecundation can take place ( ) Scopolamine abolished the secretory response day 63–68. This is a further confirmation cholinergic muscarinic nature of the response (cf. Hammarström & Sjöstrand 197

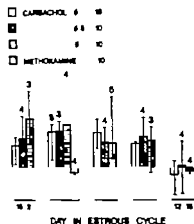
Hormone treatment (Fig. 2). Field st produced an increase in carbohydrate above resting level in uteri from animals with estrogen only (+14%). The increase however slight compared to that of uteri from animals receiving estrogen as well as prog (+49%).

No clearcut response to field stimulation tained in uteri from animals receiving progesterone only.

Effect of carbachol
(Fig. 2, 3 and 4)

Estrous cycle (Fig. 3). Carbachol (5.5× 5.5×10 M) rendered a secretory response was roughly parallel to that elicited by field stimulation (+20%). Consequently no secretory responses were seen in late diestrus (cf. above).

Pregnancy (Fig. 4). At day 6–8 of pregnancy secretory response (+20%) could be noted.



...arbohydrate release from guinea-pig uteri in re... carbachol during estrous cycle. As was the case ...d stimulation (Fig. 1) secretory responses are ...ing the whole cycle except for late diestrus. No response was noted after methoxamine at day 3–5

...o certain response was detected. This might ...to the fact that at this time rather small area ...of endometrium persists after the placentae ...membranes have been removed. Furthermore ...the resting secretion level" might conceal a ...ble secretory response (cf. below).

...mid pregnancy (day 28–42) response of about ...was noted. In late pregnancy (day 48–68) an ...greater secretory response was noted (about +60%). The secretory response persisted after delivery (+45%).

The secretory responses to carbachol ($5.5\times10^{-} - 5.5\times10^{-4}$ M) reached approximately the same level as those corresponding to field stimulation.

Hormone treatment (Fig.). In the combined treatment group as well as in the estrogen treated group carbachol raised the carbohydrate secretion to the same extent as field stimulation. Compared to the combined treatment group the response was shifted to the right by more than a power ten in the estrogen treated group.

Even very high concentrations of carbachol ($5.5\times10^{-}$ M) were without overt effect on carbohydrate secretion from uteri of animals treated with progesterone only.

Methoxamine at a concentration of 4×10^{-4} M was without overt effect on the carbohydrate secretion at day 3–5 in estrous cycle (Fig. 1).

DISCUSSION AND CONCLUSIONS

General criticism of the method has been discussed earlier (Hammarström & Sjöstrand 1979). Naturally concerning this study it should be recalled that as the endometrium definitely exhibits great functional and morphological changes according to its hormonal state a marker of secretion not subjected

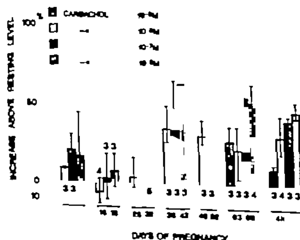


Fig. 4. Carbohydrate release from guinea-pig uteri in response to carbachol during pregnancy. Secretory responses are roughly parallel to those obtained after field stimulation (cf. Fig. 1). However, this figure also includes experiments at mid pregnancy (day 28–32 and 38–42).

to hormonal regulation is hardly possible to find Here it should be mentioned that the measured secretory responses have a draw-back constituted by the uncertainty of what "resting secretion level" actually represents e g at days 16–18 of pregnancy the high resting secretion level might disguise a secretory response

The question of what resting secretion level represents has to be touched The small variation in carbohydrate resting secretion level of uteri from animals treated with excess of estrogen or progesterone is remarkable Possibly quite a proportion of the resting secretion represents a constant carbohydrate leakage from all types of uterine tissue Concerning the lack of differences in resting secretion level between most groups it should also be recalled that changes in secretory products e g from glycogen to mucopolysaccharides may occur Such changes occur regularly in the late secretory phase in the human endometrium (Cramer & Kloss 1955 Runge Ebner & Lindenschmidt 1956 Schmidt Matthiesen 1963) Concerning the peak in resting secretion level at pregnancy day 16–18 the proportionally greater endometrial area that is occupied by placental sites should be considered The ulcered sites might render a greater leakage of carbohydrate The second maximum observed at day 63–65 possibly merely reflects the increase in size of all uterine tissue

One general conclusion seems to be justified by the results in this paper i e the responses studied are dependent on estrogen as well as progesterone Thus uteri of animals receiving both hormones showed larger responses and were also more sensitive to carbachol than uteri of animals receiving estrogen only It is well documented that both for development and maintenance of the secretory phase of the endometrium both estrogen and progesterone are necessary Yet endometrium of the guinea pig receiving excess estrogen only responded to nerve stimulation as well as to direct muscarinic stimulation This would be in line with the electron microscopical findings of e g Cramer & Kloss (1955) Wessel (1960) Wetzstein & Wagner (1960) Nilsson (1962) Wynn & Harris (1967) showing similar but minor discharge of secretory products during the proliferative phase to that occurring in the secretory phase Neither nerve stimulation nor muscarinic stimulation of the endometrium of animals treated with excess progesterone only produced any definite response in the studied parameters Whether this was solely due to [illegible]ty of secretory cells to respond to stimuli or [illegible] alterations in the neuro-effector region [illegible] vealed

The secretory responses studied roughly p[illegible] the serum progesterone level during the estr[illegible]cle and during pregnancy (Heap & Deanesl[illegible] Feder et al 1968 Challis, Heap & Illingworth [illegible] Croix & Franchimont 1975). Serum estrogen [illegible] of the guinea pig are difficult to analyze du[illegible] estrous cycle because they are very low a[illegible] are marked individual variations throughout [illegible]cle (Challis et al 1971 Croix & Franchimont [illegible] However towards end of pregnancy [illegible] serum levels are seen (Challis et al 1971) [illegible]ingly during the estrous cycle a secretory re[illegible] to nerve stimulation or carbachol could be [illegible] except for the late diestrus phase when ser[illegible] gesterone is low A secretory response to [illegible] stimulation or carbachol was present during [illegible]nancy as well as post partum The magnitude [illegible] response in late pregnancy was similar to th[illegible] uteri treated with excess of estrogen and pr[illegible]terone This makes sense since towards e[illegible] pregnancy both estrogen and progesterone [illegible] maximum levels (Heap & Deanesly 1966 C[illegible] et al 1971)

The results also suggest that the cholinergic [illegible]retory innervation persists during gestation [illegible] respect to the fact that the myometrial adre[illegible] innervation disappears during pregnancy ([illegible] 1967 Owman et al 1974 Thorbert 1978 [illegible] that uteri in late pregnancy and post partum [illegible]sponded to field stimulation points to fundam[illegible] differences between adrenergic myometrial [illegible] and endometrial cholinergic nerves

Evidence of an adrenergic possibly neuro[illegible] inhibition of the cholinergic secretory respo[illegible] transmural stimulation was obtained at d[illegible] estrous cycle Whether this inhibition is [illegible] postjunctional or both is at present difficult [illegible] Further solution of this question would req[illegible] more sophisticated method Anyhow adre[illegible] nerves to the basal endometrium of several sp[illegible] have been reported (vide e g Adham & [illegible] 1969 Dallenbach & Vonderlin 1973 Thorbert [illegible] 1977) The adrenergic innervation is depend[illegible] female sex hormones (Falck et al 1974 Owman [illegible] al 1974 Thorbert Alm & Rosengren 1978) par[illegible]larly the relative level of estrogen versus pro[illegible]terone which might be the reason for the adre[illegible]

…ry response revealed only at day 3–5 in es…le (cf. Thorbert 1978, Thorbert et al. 1978).

…It should be emphasized that secretory …s could be elicited at all crucial events of …oductive cycle of the female guinea-pig i.e. …strus, days of implantation, period of fetal …nd the end of pregnancy … well as post … when repeated fertilization naturally oc… …is conclusion is challenging and urges to …tudies concerning the significance of …e cholinergic secretory nerves.

…y grateful to Mr Jan Olof Hedborn for excellent …e in the …xperiment … to Mrs Annika Rosén for …out the pictures and to Miss Eva Svensson for …reparation of the manuscript.
…search … is supported by … grant from Svenska …et för Medicinsk Forskning and Magnus … Stiftelse.

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lar calcium as a determinant of action potential tion in rabbit myocardium

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Jóhannsson, M & Wohlfart, B Cellular calcium as a determinant of action potential duration in rabbit myocardium. Acta Physiol Scand 1980, 110 41–47 Received 28 Nov 1979 ISSN 0001-6772. Department of Pharmacology University of Iceland and Department of Pharmacology Uni ersity of Lund, Sweden

It has been suggested that the amount of calcium in the myocardial cell determines force production as well as the duration of the action potential W ha e examined tw factors proposed to determine the action potential duration (1) the inotropic state of the muscle and (2) the preceding stimulation interval. The experiments were performed on isolated papillary muscles from rabbits. The preparations were stimulated to contract isometrically at temperature of 37°C and intracellular recordings of action potential were made An increased inotropic state produced either by raised external calcium concentration or by increased stimulation frequency was associated with shortened action potentials, confirming factor (1) abov The dependence of the action potential duration on the preceding stimulation interval (factor (2) above) disappeared over wide range of interval when the preparation was exposed to 4 μM D600. The action potential duration was, however still dependent on the preceding stimulation interval when the extracellular calcium concentration had been lowered from 0 mM to 1.0 and 0.5 mM respectively The results are discussed in terms of model which postulates an inverse relationship between the amount of calcium in membrane compartment and the action potential duration.

Key ord Rabbit papillary isometric force action potentials, low calcium D600

enerally held that the intracellular concentra- f free calcium determines the contractile force ced by the myocardial cell (Ebashi & Endo Fozzard 1977) It has furthermore been sted that the intracellular calcium concentra- ontrols the duration of the action potential in ardium (Isenberg 1975 Kass & Tsien 1976 ghtwaighte Fry & McGuigan 1976, Boyett such that increased concentration of calcium cell leads to abbreviation of the action poten- n accordance with this an inverse relationship een contractile strength and action potential on has often been observed (Trautwein & l 1954 Edmands, Greenspan & Frisch 1968 & Suckel 1968 Edman & Jóhannsson 1976 tt 1978) It was proposed in a previous study lfart 1979) that the action potential duration is olely determined by the inotropic state of the le but also depends on the length of the preceding stimulation interval The influence of these two factors on the action potential was explained by assuming that a membrane compartment is gradually filled with calcium during the t me interval between stimuli and that the degree of filling inversely determines the action potential duration. The present study has been aimed at further testing this idea. To this end we have paced isolated papillary muscles with different pacing protocols in order to produce changes of the action potential duration Under these conditions we have also varied the calcium concentration in the bathing solution and compared the effects with those of the s bstance D600 which is known to block the calcium influx through the cell membrane

METHODS

Preparation and mounting Papillary muscles were dissected from the right entricles of rabbits (weight about 1 kg).

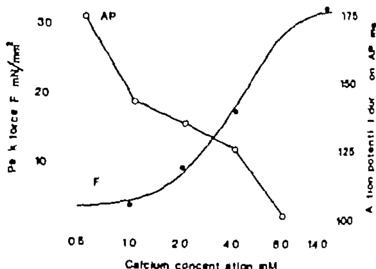


Fig 1 Peak isometric force (F) and action potential duration (AP) of an isolated papillary muscle (Paced at 1 Hz) in relation to the calcium concentration in the perfusate Note the inverse relationship between F and AP as the calcium concentration is changed

The techniques used for dissection and mounting of the muscles have been described in detail (Edman & Jóhannsson 1976) The heart was removed and split open in a warm oxygenated solution. Papillary muscles (largest diameter 0.3–0 7 mm length 3–5 mm) were excised and loops of platinum wire were tied to the tendon and to a piece of the ventricular wall at the base of the muscle The muscle was mounted horizontally in a thermostatically controlled bath (4 ml) between two hooks one of which was fitted to the lever of a force transducer The position of the other hook was controlled by means of a micrometer screw for fine adjustment of the muscle length The length was set to 95% of the length at which maximal isometric force was obtained

Force transducer Force was measured by means of a semiconductor strain-gauge transducer The free resonant frequency of the transducer was approximately 700 Hz A linear response was obtained for forces up to at least 50 mN The compliance of the transducer was less than 2 μm/mN The transducer signal was recorded on a polygraph ink writer and displayed on a storage oscilloscope (Tektronix 5103N)

Recording of membrane potential Membrane potentials were recorded by means of a conventional microelectrode technique The glass capillaries were filled with 3 M KCl and had a resistance of 20–30 MΩ The microelectrode and an Ag AgCl reference electrode were connected differentially to an amplifier with high input impedance and capacitance neutralization. The signals were displayed on the storage oscilloscope and photographed on 35 mm film. The film records were analysed at 10 times magnification The action potential duration was measured at a voltage level of −40 mV

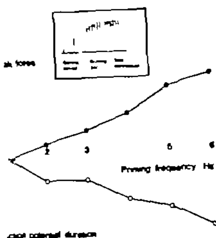
Stimulation The muscle was stimulated by passing current through a pointed electrode (platinum wire) placed over the base of the muscle The current intensity exceeded the threshold value by 30–50% Current was delivered from an electrically isolated power amplifier was operated by a Grass stimulator The stimulator gated by a programmable control unit which made it possible to pre-set different stimulation sequences ± %

Perfusion The muscle was continuously perfused min) with a bath solution of the following composition (mM) NaCl 100 0 KCl 4 0 $NaHCO_3$ 70 0, NaH_2PO_4 $MgSO_4$ 1.5 $CaCl_2$ 2.0 Na-acetate 20 0 and glucose Insulin 2 i u per liter was added. The solution equilibrated with a gas mixture containing 95% O_2 and CO_2 yielding a pH of 7 4 All chemicals used were analytical grade D600 was kindly supplied by Knoll Ludwigshafen Germany The water used for washing glassware and for preparation of solutions was distilled in borosilicate glass or Vitreosil (R) distiller The bath temperature was 36–37°C In any given experiment temperature varied by less than ±0 ·°C.

RESULTS

1 *Action potentials in relation to the inotropic state and the preceding stimulation interval*

It was reported in a previous study (Wohlfart that the action potential duration was inversely related to (1) the inotropic state of the muscle and the length of the preceding excitation interval These relations have been further examined in this study In the experiment demonstrated in Fig 1 the inotropic state of the muscle was changed by changing the calcium concentration in the bathing fluid The muscle was continuously paced at 1.0 Hz and was allowed to equilibrate for at least 15 min



ak force and action potential duration of the test ...on, marked by an arrow in the inset, related to ...lation frequency during the preceding priming ...e inset) Interval between stimuli in the control ...d before the test contraction was 1.0 s. Note the ...tionship between contractile force and action ...duration.

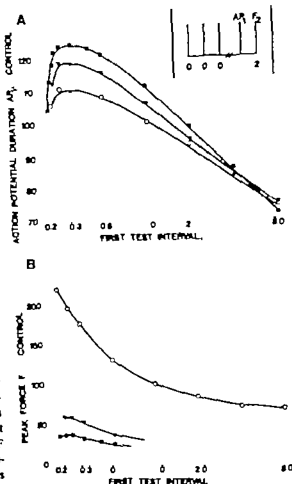


Fig. 3. (A) The action potential duration of the first test contraction (AP_1) in relation to the preceding stimulation interval. For the pacing sequence see inset and Results, section 1. The external calcium concentration was 2.0 mM (O), 1.0 mM (▲) and 0.5 mM (■). (B) Peak force of the second test contraction (F_2) after the varied stimulation interval. Pacing sequence and symbols as in panel A.

...ange of the calcium concentration. It can be ... Fig. 1 that peak isometric force (F) in... with the calcium concentration whereas ... potential duration (AP), measured at about ... (see Methods) decreased. Thus, AP and F ...d inversely as the external calcium concen... was altered. This inverse relationship was ...served during post-stimulation potentiation ...mulation sequence used is illustrated by the ... Fig. 2. The muscle was stimulated at a ...requency of 1.0 Hz. This was followed by a ... period during which a train of 16 stimuli of ... frequency was given. A test stimulus was ...ed 1.0 s after the last stimulus in the priming ... It can be seen in Fig. 2, that with increasing ...ncy during the priming period peak isometric ... F) of the test contraction increased, where... ...on potential duration (AP) at the same time ...sed.

... 3 A demonstrates how the action potential ...on is affected by changes of the preceding ...lation interval. The following stimulation ...nce (see inset of the figure) was used. The ...le was stimulated at a regular frequency of ...Hz. These control contractions are referred to ... At given moment the sequence was inter... and two test pulses, designated 1 and 2 ... ctively were applied. The first test interval, ...e the time from the last stimulus of the control period to the occurrence of test pulse 1 was varied (between 0.2 and 8.0 s). The interval between the first and the second test pulses was fixed at 1.0 s. The effects of this stimulation sequence on contractile force and action potential configuration were described and analysed in the previous study (Wohlfart 1979). Some effects will be commented on below. It is demonstrated by the open symbols in Fig. 3 A that the action potential duration of the first test contraction (AP_1) decreases as the test interval is prolonged from about 0.3 s to 8.0 s. For shorter intervals than 0.3 s, AP is reduced

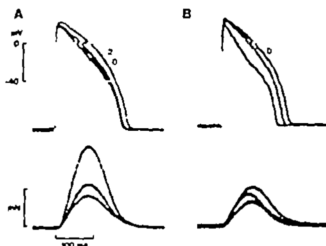


Fig 4 Oscilloscope records of action potentials and isometric myograms from the same experiment as that demonstrated in Fig 3 The external calcium concentration was 0 mM The recordings from the two test contractions (designated 1 and) are superimposed on a control record (desingated 0) The first test interval was 0.3 s in panel A and 8 0 s in panel B

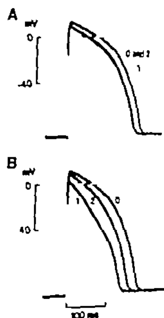


Fig 5 Oscilloscope records of action potentials [from the] same experiment as that in Fig 3 and 4 The [external] calcium concentration was 0.5 mM Numbers [refer to the] same pacing sequence as before (Fig 3A, [where] the test interval was 0.3 s in panel A and 8 0 s in panel [B]) [Note] that the changes in action potential duration [are similar to] those seen in Fig 4

The greatest changes in force production occur in the second test contraction with this stimulation sequence Fig 3B (open symbols) shows that peak force of the second test contraction (F_2) is similarly related to the interval as AP and thus reduced for the longer intervals

Fig 4 exemplifies original records of action potentials and isometric myograms of the two test contractions For comparison the records are superimposed on the control traces It can be seen that peak force of the first test contraction (F) is reduced both after a shortened (Fig 4A) and a lengthened (Fig 4B) test interval The inverse relationship between peak isometric force and action potential duration is thus not seen in contraction 1 where the nearest preceding excitation interval is varied

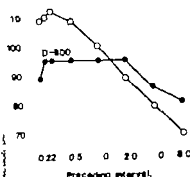
2 The effects of low calcium concentration on the relation between action potential and the preceding interval

The curve relating the action potential duration of the first test contraction (AP) to the preceding interval (Fig 3A) was shifted towards longer action potentials when the external calcium concentration was reduced from 2 0 mM to 1 0 mM and 0.5 mM This is in accordance with the results shown in Fig 1 However after the very short (about 0.2 s) and after the very long (about 8 0 s) intervals there was no significant difference between the curves in the different calcium concentrations The well known negative inotropic effect of lowering the external calcium concentration [is] seen in Fig 3B where peak force of the sec[ond test] contraction (F_2) has been related to the test [interval]

Fig 5 shows original records of action pot[entials] in an external calcium concentration of 0 5 [mM] [It] can be seen that the effects of varying the interval are similar to those seen in a calcium concentration of 2 0 mM (cf Fig 4). Thus the [action] potential of the first test contraction was [shortened] in duration after a short interval (Fig 5A) [and pro]duced after a long interval (Fig 5B) When [the] calcium concentration was reduced below 0 [5 mM] the force production was very much reduced [and] the effect was not completely reversible The[se ex]periments were therefore discarded

3 The effects of D600 on the action potential duration

The agent D600 is known to block the slow in[ward] current (Kohlhardt et al 1972 Kass & Tsien [...] Nawrath et al 1977) without any substantial ef[fect] on the duration of the action potential A concentration of 4 μM was used in the p[resent] experiments This concentration reduced



...ction potential duration of the first test contrac... function of the preceding stimulation interval ...O) and after (●) the addition of 4 μM D600 to the ... Note that the action potential duration as the ... ide range of test interval when the muscle ...osed to D600

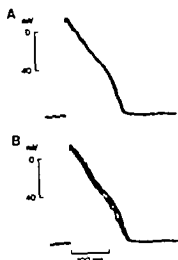


Fig. 7. Oscilloscope records of action potentials during the first (AP_1) and second (AP_2) test contraction superimposed on control record. The muscle was exposed to 4 μM D600. The pacing sequence was the same as before (Fig. 3A, inset). (A) Test interval: 0.3 s. Note that all three action potentials (control, AP_1 and AP_2) are practically identical. (B) Test interval: 8.0 s. AP_1 is slightly shortened whereas control and AP_2 are identical.

...ic force to about 10% of the control value ...20 min of exposure (stimulation rate 1.0 Hz). ...found that D600 made the action potential ...on independent of the preceding stimulation ...l over ... wide range. It can be seen in Fig. 6 ...e action potential of the first test contraction ...t change in duration as the interval was varied ...en 0.25 s and 2.0 s. The same phenomenon ...bserved in 6 other preparations. Fig. 7 demon... ...s action potential recordings of the two ...ontractions in a preparation exposed to D600 ...that the traces in panel A are identical with the ...l. The action potential recorded after a long ...al (8.0 s; panel b) is slightly shorter than the ...l (cf. Fig. 4B and 5B).

...USSION

...e action potential of mammalian myocardium is ...ated by transmembrane currents carried by ...m, calcium, potassium and probably also ...ide ions (Trautwein 1973, Noble 1975). A fast ...rd sodium current accounts for the initial rapid ...larization of the cell (Beeler & Reuter 1970 ... & Trautwein 1972). A slow inward current ...lcium ions takes place during the plateau phase ...e action potential (Beeler & Reuter 1970b ... & Trautwein 1972a and b). Sodium ions may ...participate in this current. A voltage and time ...ndent potassium current contributes to the ...repolarization of the cell. A time-independent ...ssium current has also been described that provides a constant background against which other ions move (McDonald & Trautwein 1978a and b). The action potential duration is believed to be determined by the decline of the slow inward current along with changes in potassium currents (Noble 1975, Bassingthwaighte, Fry & McGuigan 1976).

It was reported in a previous study (Wohlfart 1979) that the action potential duration was inversely related to: (1) The inotropic state of the muscle and (2) the length of the preceding stimulation interval. These relations have now been further studied and will be discussed in relation to a model which can be described as follows (cf. Edman & Jóhannsson 1976, Bassingthwaighte, Fry & McGuigan 1976, Wohlfart 1979). The amount of calcium in a membrane compartment, at the moment of excitation, is believed to inversely determine the action potential duration. When the extracellular calcium concentration is increased (Fig. 1) more calcium is taken up by the cell leading to a greater force production. At the same time the action potential duration is reduced because of the higher contents of calcium in the membrane compartment. Increasing the stimulation frequency (Fig. 2) represents another way of loading the cardiac cell

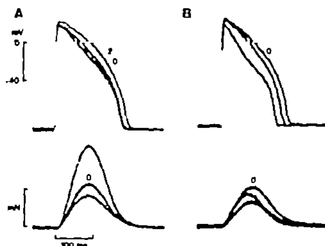


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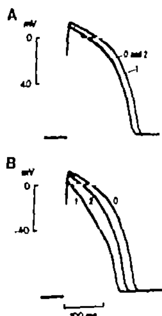


Fig 5 Oscilloscope records of action potentials ... same experiment as that in Fig. 3 and 4 The ... calcium concentration was 0 5 mM Numbers ... same pacing sequence as before (Fig. 3 A, ... test interval was 0.3 s in panel A and 8.0 s in panel ... that the changes in action potential duration ... those seen in Fig 4

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with calcium It should be noted however that changes in stimulation frequency will also affect the action potential duration via factor (2) i e the length of the preceding interval The influence from this factor can be kept constant by using the protocol described for Fig 2 Peak force and action potential duration are here related in a test contraction that is elicited at a fixed interval after a priming period with different stimulation frequencies The figure shows that the increased inotropic state provided by raising the stimulation frequency is associated with shortened action potentials

In order to explain the influence of the preceding stimulation interval per se upon the action potential duration additional assumptions have to be made (1) Calcium is released from the membrane compartment during the action potential (2) The membrane compartment is gradually replenished by calcium during the interval between action potentials An action potential elicited after a long stimulation interval (Fig 3 A and 4 B) will therefore be of a short duration The abreviated action potentials which are seen after very short intervals (shorter than 0 3 s in Fig 3 A) may be explained by an overriding influence of a slow recovery from inactivation of the slow inward current (Gettes & Reuter 1974) and/or by a persisting outward potassium current (Hauswirth Noble & Tsien 1972)

The proposed model is compatible with the findings reported here Lowering the external calcium concentration from 2 0 mM to 1 0 mM and 0.5 mM changed the relation between action potential duration and the preceding interval (Fig. 3 A) towards longer action potentials The curves in Fig 3 A seem to meet however for the very short (about 0 2 s) and the very long (about 8 0 s) intervals In the former case this may be due to the refractoriness of the ion mechanisms behind the action potentials discussed above as the interval is shortened below 0 3 s Action potentials elicited after the very long intervals may be independent of the external calcium concentration because the membrane compartment has been gradually loaded with calcium towards the same final filling level

The compound D600 has been reported to act on the cell membrane and thereby inhibiting the slow inward current (Kohlhardt et al 1972 Kass & Tsien 1975 Nawrath et al 1977) This would result in a reduced cellular uptake of calcium and a negative inotropic effect The duration of the action potential is however not affected to any great extent It has been shown in the present study that D6[illegible] the action potential constant in duration [illegible] range of preceding test intervals (Fig 6) Th[illegible] of D600 does not seem to be a co[illegible] reduction of activator calcium in the cell [illegible] relation between action potential duration [illegible] preceding interval (Fig 3 A) was still pre[illegible] lowering the calcium concentration in the [illegible] solution In terms of the presented model [illegible] reasonable that D600 inhibits the coupling [illegible] the membrane compartment and the action [illegible] tial duration High external calcium concen[illegible] have been shown to neutralize the inhibitor[illegible] slow inward current by D600 (Kohlhardt [illegible] 1972) The reduction of the action potentia[illegible] tion that was seen after the long stimulation [illegible] vals in the presence of D600 (Fig 6) may be due to increased calcium contents in the [illegible] brane compartment which overcomes the [illegible] tion by D600 (cf Lewartowski Prokopczuk [illegible] kowski 1978) The reduction of the action [illegible] duration after the short intervals may [illegible] above) be attributed to the action potential[illegible] elicited in the relative refractory period.

The action potential duration and [illegible] of the muscle seem to be mutually related [illegible] crease in the action potential duration [illegible] assumed to increase the cellular uptake of c[illegible] and therefore raise the inotropic state of the [illegible] (Antoni Jacob & Kaufmann 1969 Wood [illegible] & Weidmann 1969 Braveny & Sumbera [illegible] creased contents of cellular calcium, on the [illegible] hand will shorten the action potential. [illegible] intracellular calcium controls both the [illegible] force and the action potential duration [illegible] mechanism may serve to stabilize the co[illegible] state of the muscle

The authors wish to thank Professor K A P E[illegible] helpful discussions and for criticism of the m[illegible] and Dr M I M Noble for correcting the manuscr[illegible] drawing of the pictures by Miss Eva Hansson [illegible] appreciated The work was supported by grants [illegible] Swedish Medical Research Council (project [illegible] 184) from the Medical Faculty of the University [illegible] and from the Icelandic Science Foundation

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siol Scand 1980, 110: 49–58

bution of blood flow in the dog kidney

tion rates of inert diffusible tracers versus uptake of 15 μm microspheres
sodilation and vasoconstriction

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CLAUSEN G. HOPE, A KIRKEBO A. TYSSEBOTN I & AUKLAND K. Distribution of blood flow in the dog kidney II Saturation rates of inert diffusible tracers ersus uptake of 15 μm microspheres during vasodilation and vasoconstriction. Acta Physiol Scand 1980 110: 49–258 Received 24 Jan 1980 ISSN 0001-6772. Institute of Physiology University of Bergen Norway

While 15 μm microspheres (M) in principle provide measure of glomerular flow uptake rate of inert diffusible tracers indicates effectiv or nutrient" flow i.e. essentially postglomerular capillary flow Paired measurements of glomerular and postglomerular flow ere made in tissue samples from outer middle and inner cortex (C C_i, C) and medullary zones. After control M injection, renal vascular conductance (RVC) was altered in one kidney whereupon a second M injection was made, immediately followed by infusion of the ^{125}I-iodoantipyrine and tritiated water RVC was increased maximally by a infusion of acetylcholine ith and without reduced renal arterial pressure whereas moderate asodilation was produced by lowering renal arterial or rising ureteral pressure. RVC was reduced by i.a. or infusion of angiotensin II Within mean RVC range of 50 to 180 % of control the fractional distribution of zonal postglomerular flow remained unaltered in agreement with previous results obtained from local H gas desaturation rate. Glomerular flow was about 20 % higher in C equal in C_i and 40 % lower in C_i as compared to postglomerular flow in control kidneys. This disparity nearly disappeared during maximal asodilation and tended to increase during vasoconstriction. The results might suggest variable net postglomerular effective flow in radial direction through the renal cortex. Alternatively the fractional redistribution of M might reflect variable degree of Ms skimming at the afferent arteriolar inlets along the interlobular arteries.

Key ords Renal blood flow kidney circulation, local flow measurement, microspheres, inert diffusible tracers, vasodilation, vasoconstriction

istribution of renal blood flow among outer ner cortical layers has been the subject of a number of studies. Several recent reviews ere 1975 Stein 1976, Aukland 1976, 1980 ar et al 1977) leave no doubt that dological differences and inadequacies have ned the issue—and still do The present work des paired measurements by two of the most monly used methods, both with a record of reproducibility but known to give different ts in the dog kidney

en renal vasodilation is induced by lowering l arterial pressure raising ureteral or renal ven- pressure or by infusion of vasodilator substances, 15 μm microspheres (Ms) invariably ind cate markedly greater increase of vascular conductance in inner than in outer cortex. On the other hand, no such redistribution has been demonstrated in comparable studies with methods based on local uptake or washout rate of inert diffusible flow indicators (DI) in anatomically well defined cortical zones During renal vasoconstriction induced by occlusion of the carotid arteries Ms indicate a greater fall of conductance in inner than in outer cortex (Banks 1975 Prosnitz 1977), but vasoconstrictor substances do not seem t produce a similar effect. The DI techniques have consistently failed to demonstrate fractional redistribution of renal blood

Acta Physiol Scand 1980, 110: 249–258

[Distri]bution of blood flow in the dog kidney

[II. Satura]tion rates of inert diffusible tracers [v]ersus uptake of 15 μm microspheres [during va]sodilation and [va]soconstriction

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CLAUSEN, G., HOPE, A., KIRKEBO, A., TYSSEBOTN, I. & AUKLAND, K.: Distribution of blood flow in the dog kidney. II. Saturation rates of inert diffusible tracers versus uptake of 15 μm microspheres during vasodilation and vasoconstriction. Acta Physiol Scand 1980, 110: 249–258. Received 24 Jan. 1980. ISSN 0001-6772. Institute of Physiology, University of Bergen, Norway.

While 15 μm microspheres (Ms) in principle provide a measure of glomerular flow, uptake rate of inert diffusible tracers indicates "effective" or "nutrient" flow, i.e. essentially postglomerular capillary flow. Paired measurements of glomerular and postglomerular flow were made in tissue samples from outer, middle and inner cortex (C_1, C_2, C_3) and medullary zones. After a control Ms injection, renal vascular conductance (RVC) was altered in one kidney. Thereupon a second Ms injection was made, immediately followed by infusion of the ^{125}I-iodoantipyrine and tritiated water. RVC was increased maximally by i.a. infusion of acetylcholine with and without reduced renal arterial pressure, whereas moderate vasodilation was produced by lowering renal arterial or rising ureteral pressure. RVC was reduced by i.a. or i.v. infusion of angiotensin II. Within a mean RVC range of 50 to 180% of control the fractional distribution of renal postglomerular flow remained unaltered, in agreement with previous results obtained from local H_2 gas desaturation rate. Glomerular flow was about 20% higher in C_1, equal in C_2 and 40% lower in C_3 as compared to postglomerular flow in control kidneys. This disparity nearly disappeared during maximal vasodilation and tended to increase during vasoconstriction. The results might suggest a variable net postglomerular effective flow in radial direction through the renal cortex. Alternatively, the fractional redistribution of Ms might reflect a variable degree of Ms skimming at the afferent arteriolar inlets along the interlobular arteries.

Key words: Renal blood flow, kidney circulation, local flow measurement, microspheres, inert diffusible tracers, vasodilation, vasoconstriction

[The dis]tribution of renal blood flow among outer [and in]ner cortical layers has been the subject of a [large n]umber of studies. Several recent reviews [(Sp...]ere 1975, Stein 1976, Aukland 1976, 1980, [...]re et al. 1977) leave no doubt that [metho]dological differences and inadequacies have [confu]sed the issue—and still do. The present work [provi]des paired measurements by two of the most [comm]only used methods, both with a record of [good] reproducibility but known to give different [resul]t in the dog kidney.

[Wh]en renal vasodilation is induced by lowering [rena]l arterial pressure, raising ureteral or renal venous pressure or by infusion of vasodilator substances, 15 μm microspheres (Ms) invariably indicate a markedly greater increase of vascular conductance in inner than in outer cortex. On the other hand, no such redistribution has been demonstrated in comparable studies with methods based on local uptake or washout rate of inert diffusible flow indicators (DI) in anatomically well defined cortical zones. During renal vasoconstriction induced by occlusion of the carotid arteries, Ms indicate a greater fall of conductance in inner than in outer cortex (Banks 1975, Proanitz 1977), but vasoconstrictor substances do not seem to produce a similar effect. The DI techniques have consistently failed to demonstrate fractional redistribution of renal blood

flow among well defined cortical zones during vasoconstriction Thus local blood flow estimated by Ms and by DI would appear to respond differently to changes in renal vascular conductance However failure to detect fractional flow redistribution during vasodilation or constriction might at least in part be ascribed to the methodological inadequacies of previously employed DI techniques as reviewed by Aukland (1976 1980*b*) For example in the case of the local H_2 gas washout technique tissue trauma induced by electrode insertion might cause erroneous measurements In any case comparison of Ms and DI results from different laboratories seems rather hazardou due to varying definitions of renal zones varying Ms sizes and to inherent basic differences between various DI techniques However there can be no doubt that Ms and DI do differ with respect to local flow in outer and inner cortical zones during control conditions as directly demonstrated by paired measurements (Clausen et al 1979*a*)

Whereas Ms are extracted from the blood stream by the glomeruli uptake or washout of DI takes place mainly through the peritubular network of exchange vessels Thus the effective or nutrient flow with respect to the particular DI used gives a measure of postglomerular capillary flow Since this capillary network is a low pressure vascular compartment (5–15 mmHg) minute changes in its interzonal resistance or pressure relations might alter the relationship between the glomerular and postglomerular flow pattern and possibly affect renal excretory function We therefore designed the present experiments to obtain paired comparison of local flow estimated from uptake rate of two DI and from 15 μm M uptake in zonal tissue samples during experimental conditions known to alter intrarenal Ms distribution

Preliminary reports of parts of this study have been publi hed (Clausen et al 1978 Aukland 1978)

MATERIAL AND METHODS

The experiments were made on 45 mongrel dogs of 15–.5 kg b wt fasted over night but w th free acces to water Anesthesia was induced by i v injection of Mebumal .5 mg/kg b wt and maintained by doses of 3–5 mg/kg as needed Free airways were maintained by an endotracheal tube without assistance of entilation Isotonic saline was infused i v at a rate of 2–4 ml/min to maintain a positive water and salt balance Lower aortic blood pressure (AP) was recorded with Hewlett Packard transducer and recorder One or both kidneys were exposed [illegible] toneally and nylon snares loosely placed [illegible] pedicles Renal blood flow (RBF) was record[ed] magnetically with a Nycotron probe on the [illegible] the experimental kidney Radiolabelled Ms [illegible] into the left ventricle through a catheter [illegible] right carotid artery Reference blood samples [illegible] a rate of 1 ml/min from an aortic catheter [illegible] femoral artery to the level of the renal [illegible] prior to and lasting 45–60 s after completed Ms [illegible] The first Ms injection, Ms_1 was made during [illegible] and AP control conditions. Then vasodilation or [con]striction was produced by procedures desc[ribed] After 3–5 min with steady RBF and AP the sec[ond] injection Ms_2 was made Within 3 min after [illegible] tion constant rate i v infusion of radiolabelled [dif]fusible flow indicators (DI) lasting for 8–18 s was made [with] serial arterial blood sampling from the aortic cath[eter] renal pedicles were occluded by tightening the [snares] before ending the DI infusion. The kidneys [illegible] were rapidly excised frozen in CO_2-ethanol [and stored at] −70°C until dissection

From a 5–7 mm thick sagittal slice cut by [illegible] frozen kidney about 10 radial sectors were [cut] Each sector was divided int three cortical [zones of equal] thickness (C_1 C_2, C_3) and two outer med[ullary zones] (OM_1 and OM_2). Details on zonal identification [and] counting of radioactivity in tissue and blood [samples have] been described in a previous paper (Clausen et al [illegible]

The Ms batches had average diameters [illegible] 13 0 (S D 1.5) to 16.1 (S.D 4) μm, as measured [micros]copically and were labelled with [141]Ce and [85]Sr [illegible] pany) However the mean diameters of Ms_1 and [illegible] equal in each experiment The diffusible [flow indicators] were mixed in 15 to 20 ml isotonic saline [illegible] [125]I iodoantipyrine (I Ap) and 50 mCi of tritiated [water] (THO) and infused at a constant rate of about [illegible]

Calculation of local flow by DI was made [according to] the method of Kety (1951) (For details and [illegible] discussion of the methods, see Hope et al 197[illegible] et al 1979*a* 1980) Vasodilation or vasoconstriction [with] and without altered renal perfusion pressure [was produced] according to the following protocol

Group 1 n=10 After Ms injection, [acetylcholine] (Ach) was infused at a constant rate into one [renal artery] through a thin polyvinyl tube inserted retrograd[ely] [illegible] blood stream The infusion rate was adjusted to [give a] 50 to 100% increase of RBF before making the [illegible] infusions

Group 2 n=6 Ach was infused as in group 1 [illegible] injection RBF was thereafter reduced to [illegible] Ach level by means of an adjustable clamp on the [renal] artery before the Ms and DI infusions Renal [arterial] pressure (RAP) was recorded via a cannula [illegible] the renal arterial catheter and was not affected [by the] present volume infusion rates

Group 3 n=1 After Ms injection, angiotensin (Ang) wa infused into the renal artery (n=4) or [into a] femoral vein (=6) at rates adjusted to give a [illegible] decrease of RBF before the M and DI infusions

Ach and Ang di solved in isotonic saline w[ere infused] i a at rates of .5–25 μg and 0 1 1 0 μg/[min]

Renal scular conduct nce (RCV) calculated from renal perfusion pressure and renal blood flow ed by microspheres (M) nd diff sible flow indicators (DI) during vasodilation by acetyl holi e duced renal rterial pressure (RAP) and elevated ureteral pressure (UP) and duri g asoconstric ngiotensin II (A g) th and thout elevated arterial pressure (AP)

4 kidneys, E exptl. kidneys

	Control period		Exptl. period				
	Pressures (mmHg)	RVC (µl/min g mmHg)		Pressures (mmHg)		RVC (µl/min g mmHg)	
	Ms_1	Ms_1		Ms_2	DI	Ms_2	DI
AP	129±17	28.8		126±18	123±16	31.2	31._
AP	129±17	29 4	Ach	126±18	123_16	52.5	50.6
AP	136±17	24 9		134±19	137±18	17.3	15 7
RAP	134±17*	47.9*	Ach RAP↓	58±6	58+6	49.0	51 1
AP	129±10	31 4	Ang i.a.	129_10	135±10	1.8	24.3
AP	137_12	27 6	Ang	217±3_	213±37	9.8	11
AP	142±14	26 7		145±15	145_15	23.8	25 1
RAP	133±17	25 9	RAP↓	75±7	72±15	43.5	50.4
AP	125±14	25 3		127±14	177±14	24 4	25 6
AP	125±14	23 5	UP↑	98±18*	98±18*	35.8	35.8

ffered during control and xptl periods
; sion pressure calculated as AP−(UP 20) mmHg with UP=49 17 mmHg.

Intravenous infusion rate of Ang was 5–100 ng/

4 19 After Ms_1 injection, renal arterial pres- s lowered to 60–80 mmHg by an adjustable clamp renal artery before the M and DI infusions

5 7 After Ms_1 injection ureteral pressure is raised to about 40 mmHg by elevating the outlet heter inserted into the renal pelvis via the upper

age local blood flow ($\widetilde{RBF}$) ml/min g was calcu- om zonal local flow rates and the respectiv zonal taken to be C 29 C 23 C_3 18, OM 12, OM 8.5 kidney eight (Clausen et al 1980) The elec- etically recorded RBF served only as indicator for d changes and to make sure that steady flow was ed during tracer administration (correct zero flow thout exact probe calibrations)

ascular flow measured with M includes flow perfuses the vasa recta and the medullary capil- Intrarenal flow distribution is therefore presented fraction (A) of total RBF obtained in C_1 C_2 and C_3 he medulla (C_3M) zones Zonal fractions obtained s_1 and Ms_2 were compared by Student' -test for d data. With DI, here only one measurement was in each kidney the zonal flow fractions of experi- l and control kidneys were compared by -test for red data. The data are presented as mean ± S D <0 05 considered significant.

al vascular conductance (RVC) was calculated as /AP µl/min g mmHg in groups 1 and 3 or as $\widetilde{RBF}$/ in groups 2 and 4. The reduction of perfusion pres-

sure caused by elevating UP was taken to be UP minus 20 mmHg

RESULTS

Average mean arterial pressure (AP) did not differ significantly among the groups during control con- ditions (Table 1). No consistent changes of AP were observed during the experimental interventions except during nfusion of Ang. Mean renal ar terial pressure (RAP) recorded during control was 7 (0–20) mmHg lower than AP measured in the lower aorta. During constriction of the renal artery (groups 2 and 4), RAP remained fairly stable but was on average 2.5 (+9 to −6) mmHg lower during DI as compared to Ms infusion the difference being statistically not significant

Individual results are presented for group 1 vas- odilation by Ach only Fig. 1 demonstrates propor tionality between local flow in the zones and $\widetilde{RBF}$ with the two DI in control and experimental kidneys over wide range of RBF With Ms and Ms_2 in experimental kidneys highly significant linear cor relations were obtained between zonal flow rates and $\widetilde{RBF}$ In contrast to DI the Ms showed that C flow increased relatively les C_3M flow relatively

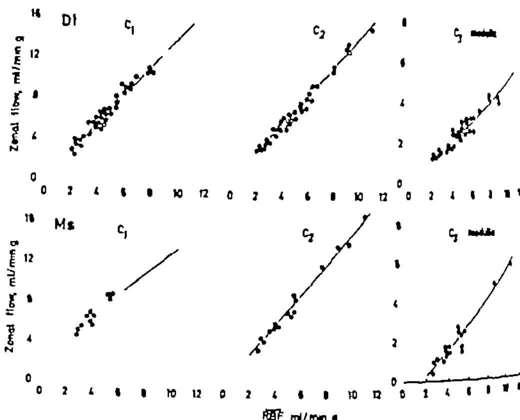


Fig 1 Local flow with diffusible indicators (DI) and with microspheres (Ms) related to average renal blood f... in 10 dogs during i a. Ach infusion C C, and C_3M Outer middle cortex and Inner cortex + medulla Filled ... circles. Control and experimental kidneys respecti ely The regression equations for local flow (F

	DI	Ms
C	F=1 40 RBF+0 24 r=0 96	F=1 09 RBF+ 30 r=0 94
C	F=1 50 RBF−0 5 r=0 98	F=1 61 RBF−0 63 r=0 98
C_{3}	F=0.50 RBF+0 16 r=0 88	F=0 68 RBF−1 5 r=0.96

more than RBF However the relationship between C_3 flow and RBF wa closely similar with Ms and DI as seen from the regression equations of Fig 1

The average zonal flow fractions and RBF obtained by I Ap and THO in all groups are listed in Table 2 The C_3 fractions were not significantly different with the two indicators However the C M flow fractions were slightly higher with THO on average 1 8±0 8% of RBF ($P<0$ 05) and the C fractions slightly lower 1 4±0 6% of RBF ($P<0$ 05) RBF tended to be lower with THO but the difference was statistically not significant

The most conspi ious observation was that no significant change of the zonal flow fractions occurred in any group with either of the two diffusible indicators

During maximal dilation induced by Ach and with RAP lowered to about 60 mmHg RBF averaged about 2 9 ml/min g A slightly lower average 2 3 ml/min g was obtained during i v infusi... with RAP averaging 217 mmHg Further... erage RBF was about 160% higher during ... during i a infusion of Ang while RAP re... control level in both groups. Thus marked ... of either RAP or RBF did not per se alter th... fractions of effective blood flow

Microspheres

Table 3 lists the average zonal flow fracti... RBF obtained by Ms in all groups Compar... zonal fractions with Ms and Ms_3 in control ... showed no significant differences Furthermore ... average zonal flow fractions and RBF wi... the experimental kidneys i e during control ... tion were not significantly different from c... kidney values with the exception of group ... below) Thus the reproducibility of the Ms m... should be satisfactory for detecti...

onal fractions of total RBF and average renal blood flow (RBF) determined by two diffusible

outer and middle cortex, C_4M inner cortex+medulla, C=control kidney

¹³¹I-iodoantipyrine (% of RBF)			RBF (ml/min g)	Tritiated water (% of RBF)			RBF (ml/min g)
C_1	C_4	C_4M		C_1	C_4	C_4M	
42 5 3 3	32 6 1 7	24.9±3 5	3 90_1 3	40.3_3 7	32.9±2.0	26.8±4	3 78±1.8
41 3±3 2	33 3_1 3	25 4_3 0	6 40±2 0	39.8 5 4	32 6±3 0	26.3±4	6.35+2.3
44 2±3 5	32 7±1 4	23 3±3 9	_32±0 7	41 7 2.1	32 8±0.8	25.6±2 6	1 99±0 7
42 0 3 0	33 0+0 9	4 1±3 7	3 25±0 9	40 0±3.5	33 _1.5	26 8_3.0	68+0.7
42 5±3 6	33 9±3 4	4 4±4 2	2 69±1 0	40 6±2.5	32.5±2.2	26 9_3.8	_23±0 7
41 6 3 2	32 7 1 2	25 7_3	3 89±0 9	39 5_3.2	3' 6_2 0	77 7±4 1	3.37_0 4
40 6+4 8	32 8±2 0	26 7±5 8	3 65±0 8	39.6±4 8	33 5_1 1	26.9_3.3	3 63±0 9
43 1 3 3	31 7±1 7	25 1±3.3	3 33±1 2	42.1±4 3	30.5 1 7	27 4_3 7	3 18±1 2
44 8±3 3	33 1±_0	22 3±4 0	3 5'+0 7	43 ±2 8	33 0_2 6	23 8_4 0	3.51±1 0

n inter zonal M distribution during vasculation and constriction. Total renal vascular ance averaged 77 1 with Ms (Table 1) with ation among the groups (S D _0) except xperimental kidney of group 2 where Ach sed i.a. during Ms_1 The Ms_1 results in experimental kidneys agree well with those by Ms_4 in group 1 (Table 3) both reflecting t of Ach infusion However group 2 conneys differed markedly with respect to Ms tribution, even though RVC was in the nor e (Table 1), and average $\overline{RBF}$ fell from 3.4 2.3 (Ms_4) contrary to the controls of the oups It should be emphasized that superimposed on Ach infusion, RAP was lowered to well below the autoregulatory range (Table 1) and accordingly that experimental kidney weight averaged 85 (S D 8.5)% of control kidney weight.

A consistent observation was that the C_3 flow fraction, closely similar to that obtained with DI remained practically unchanged both in control and experimental kidneys in all groups (Table 3) Thus the changes in zonal flow fractions may be illustrated with reasonable accuracy as the changes of the C or C_4M flow fractions. Fig. depicts the relationship between changes in C_4M flow fraction and RVC During vasodilation C_4M flow increased proportionately more than $\overline{RBF}$ while the opposite

Zonal fractions of total RBF and average renal blood flow (RBF) determined by micro spheres

outer and middle cortex C_4M inner cortex medulla, C=control kidney E=experimental kidney

Control period, Ms_1 (% of RBF)			(RBF) (ml/min g)		Exptl period, Ms_4 (% of RBF)			(RBF) (ml/min g)
C_1	C_1	C_4M			C	C_4	C_4M	
51 4 3 9	32 5±3	16 1+4 7	3 72±1.2		50 8±7.5	33.3±4 6	16 0±4 8	3 94±1 5
51 0±6 6	32 8±3 3	16 2±4 5	3.80±1 0	Ach	42.0±4 9	34 8±3.4	23 2±4.8	6 6_±2 2
60 3±6 7	28 7±3 7	11 1±3 4	3 39±1.5		60 0±6 3	29.3±3 9	10 3±2 8	_32±0 7
43 3 3 8	35 5±1 5	21 1±5 1	6.51±2.7	Ach RAP↓	42 5±4 4	37 0±1.2	20 6±4.8	2.84±0 7
51 6_5	32 4±2.7	20 0±4 6	3 91±1 5	Ang	52.6±6 5	33.9±2.3	13.5±5 4	2.36±0 9
52 4 7	31 0±_5	16 8±4.5	3 77±1.3		53 4±5.0	30.2±3 1	16 4±4 4	3.45±1 1
49 7 4 2	31 4±3 1	18 9±3 8	3 50±1 1	RAP↓	43 6±4.0	33.8±3 0	22.6±4 9	3.26±0 8
49 0_5 2	33 7±_9	17.3±5 0	3 16±0 6		49 1±4.5	32 6±3.0	18.4_3.5	3 10±0 7
49 7±6 5	33 4 3 9	17.0±4 3	2.94±0.5	UP↑	46 7±4 0	35 1±4 0	18 1±3 9	3 49±0 7

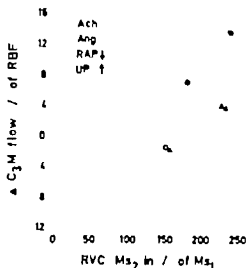


Fig Changes in the fraction of total RBF perfusing inner cortex and medulla (C_3M) as related to renal vascular conductance (RVC) in experimental kidneys.

pattern was observed during vasoconstriction The corresponding relative changes in intrarenal resistance blood vessel diameters are −30 to +25% calculated as the percentage change of $\sqrt[4]{\overline{RVC}}$ from Fig 2 data grouped in 40% RVC intervals

Comparison of DI and Ms results

Average $\overline{RBF}$ obtained by the two techniques (Tables 2 and 3) did not differ significantly in any group Since the C flow fractions obtained by Ms and DI were closely similar in control and experimental kidneys and among the groups relative changes in the flow distribution patterns will be reflected by the respective C M fractions as summarized in Fig 3 Vasoconstriction increased the difference whereas vasodilation markedly reduced the difference between the C M flow fractions obtained by DI and Ms

Elevation of UP produced a moderate increase of RVC as compared to the other vasodilatory interventions (Table 1) The reduced difference between C M fractions obtained by Ms and DI was due to a lower C_3M fraction with DI in the experimental kidney ($P<0.05$) and not to increased C_3M fraction with Ms as in the other vasodilation groups $\overline{RBF}$ averaged 3.5 ml/min g with both Ms and with DI but only 2.9 ml/min g with Ms in the experimental kidney (Tables 2 and 3) Thus total RBF increased by 19% However since left kidney exptl weight averaged 118 (S D 8)% of right kidney weight $\overline{RBF}$ did probably not increase during elevation of UP

DISCUSSION

Flow distribution among cortical zones

The results obtained with two inert diffusible tracers clearly indicate that zonal fractions of C[illegible] not change significantly during marked acute decrease of total renal vascular conductance [illegible] agrees well with previous results obtained by local H_2 gas clearance method during vasoc[illegible] produced by the same experimental means as in the present groups 1 and 2 and during constriction by i.a. infusion of catechol[illegible] (Tyssebotn & Kirkebo 1975 1979).

Reduction of RAP within the RBF autoreg[illegible] range produced no changes in zonal flow [illegible] with DI This again agrees with local H ga[illegible] ance studies (Loyning 1971) Elevation of U[illegible] duced a slight decline in the C_3M flow fra[illegible] Comparable studies have apparently not bee[illegible] with methods based on the uptake or wash[illegible] of inert diffusible tracers in well defined ana[illegible] regions of the dog kidney

Also the results obtained with 15 µm Ms [illegible] fairly well with previous observations by (using the same technique Thus a relative [illegible] of deep cortical flow during Ach dilation [illegible] reported by McNay & Abe (1970a), Stein (1971) Abe et al (1973a) and Baehler et al while Rector et al (1972) observed a 20% [illegible] cally not significant reduction in the deep c[illegible] flow fraction during vasoconstriction by A[illegible] average changes of RVC in these studies we[illegible] lar to those obtained in the present exper[illegible] Reduction of RAP has been found to increase cortical flow fraction as determined by Ms (M[illegible] & Abe 1970a b Abe et al 1973a b). Eleva[illegible] UP to 50–60 mmHg had the same effect (Ba[illegible] 1972 Abe et al 1973b)

A more detailed comparison of the intrarenal patterns obtained in the present and pr[illegible] studies cited above does not seem fruitful [illegible] methodological differences such as the use of 4 cortical zones Less obvious are the [illegible] quences of allowing the kidneys to drain [illegible] measuring tracer concentrations and of somewhat different Ms sizes For example a 20% volume increase as observed during [illegible] by Ach (Omvik et al 1971) would produce a 20% decrease in local H gas clearance [illegible] other hand kidneys allowed to drain before during dissection a [illegible] if not all Ms [illegible]

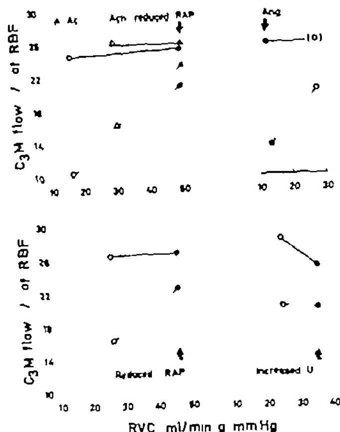


Fig. 3. The fraction of total RBF perfusing inner cortex and medulla (C_3M), as related to renal vascular conductance (RVC). Solid lines: diffusible indicators. Broken lines: microspheres. Open symbols: control kidneys.

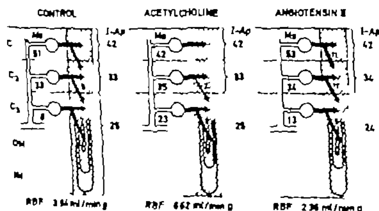


Fig. 4. Hypothetical net inward postglomerular effective flow of blood accounting for the difference between zonal flow obtained by microspheres (Ms) and diffusible indicator (I–Ap). The numbers give flow in % of total RBF.

cited above may loose 10 to 20% of their volume As a result local flow per g tissue will be overestimated Interzonal volume shifts that may occur in the present undrained and rapidly frozen kidneys are probably negligible and Ms and DI data will be directly comparable A disproportionate increase of volume in deep renal zones may well occur during elevated UP as suggested by Szabo et al (1976) This would explain why the C_3M flow fraction fell with DI and failed to increase with 15 μm Ms (Fig 4)

Microspheres somewhat larger than 15 μm may be subject to purely steric restriction or hindrance from entering afferent arterioles in the dog kidney according to the results of Morkrid et al (1976) Thus the redistribution of 19 μm Ms during elevated UP (Bay et al 197) could be due partly to increased arteriolar diameter partly to a greater increase of RVC produced by a UP higher than the present average Similarly steric restriction for 15 μm Ms during a 20 to 30% reduction of resistance vessel diameter cannot be excluded It is worth noting that where actually measured in earlier studies the mean Ms diameter turned out to be about 18 or 19 μm (McNay & Abe 1970*a* *b* Bay et al 1972)

With these methodological reservations in mind we conclude that the present results confirm both the previous H_2 gas clearance and Ms studies and directly demonstrate what previously might be inferred from separate studies from different laboratories. 15 μm Ms may be redistributed among outer and inner cortical zones in spite of maintained distribution of effective or nutrient flow in the dog kidney Accordingly failure to detect fractional flow distribution between inner and outer cortex by the local H gas washout method cannot be ascribed to local tissue trauma around the inserted electrodes

Interpretation of the disparate flow distribution patterns obtained with DI and Ms

It has been repeatedly suggested that redistribution of blood flow among superficial and deep nephrons may be of importance for the regulation of renal salt and water excretion as reviewed by Lameire et al (1977) However so would probably changes in the relationship between zonal glomerular and postglomerular flow i e between filtering and reabsorbing flow The present results indicate that this relationship may be changeable [illegible] able postglomerular capillary flow [illegible] tion However this concept rests [illegible] er the present tracers are valid [illegible] merular flow (Ms) and postglomerular [illegible] flow (DI) as discussed below

Diffusion of DI directly from the [illegible] *teries* (i l.a) to C_3 tissue would imply that [illegible] or nutrient blood flow was higher than [illegible] blood flow to this zone and might be [illegible] the disparity between Ms and DI [illegible] served in control kidneys. However [illegible] made in groups I and exclude this [illegible] possible explanation When RBF is [illegible] measured DI concentration difference [illegible] i l a blood and C tissue is increased

Closely similar RVC values were obtained [illegible] groups during Ach infusion (Table 1), [illegible] RBF was reduced from 6.5 to 2.9 ml/[illegible] 3) suggesting unaltered i.l.a. diameter [illegible] neither diffusion gradient nor surface [illegible] which diffusion might take place were [illegible] indicating that the absolute amount of DI [illegible] up by C_3 directly from i l.a. was [illegible] increased

Fractional arteriolar inflow to C_3 was [illegible] as RBF was reduced as indicated by Ms [illegible] Thus if an appreciable portion of the DI [illegible] was derived directly from the i l.a. the C_3 [illegible] fraction should be markedly higher in [illegible] in group I experimental kidneys Since this [illegible] the case (Table 2) we conclude that direct DI [illegible] by diffusion from the deep portion of the [illegible] negligible

The mixing effect of blood flow in the [illegible] *network* would tend to reduce interzonal [illegible] centration differences and accordingly [illegible] create an inward effective flow of DI from C_1 but not from C to C where on average [illegible] gradient exists This also applies to the only [illegible] cortical inward postglomerular flow of [illegible] that taking place in interlobular [illegible]

Thus neither the mixing effect or inward [illegible] flow nor direct diffusion from the i l.a. [illegible] the variable disparity of Ms and DI flow [illegible] tion

A net inward postglomerular flow in the [illegible] during control conditions was proposed in [illegible] ing reports (Clausen et al 1978 1979*a*), as [illegible] the generally accepted concept that 15 μm Ms [illegible] a true estimate of glomerular flow and that [illegible]

…l to the tissue essentially through post-…ar capillaries. Fig. 4 illustrates how this net …mponent according to the present results … during vasodilation and increases during …striction. This model requires that 70% of …nal blood perfusing C glomeruli during … conditions attains DI transcapillary diffu-…librium on average 0.5 to 1 mm deeper than …st glomerulus. The better agreement be…Ms and DI observed during vasodilation im-…at capillary blood now equilibrates with …nd drains to the interlobular veins at the …el as the parent glomerulus. This shift … consequence of the disproportionate in-…of vascular conductance in the C arterioles …ated by the Ms data. The resulting increase …glomerular capillary pressure in C would …create a net outward flow of blood through …et. We have previously presented a model …ing this effect (Aukland 1978) and provided …dependent experimental evidence for a var…et cortical postglomerular flow (Aukland …

…ning of 15 μm Ms at the arteriolar inlet … could be another major—if not the …eason for the disparity of the Ms and DI flow …s in control kidneys. This is suggested by … findings indicating that 15 μm Ms underesti-…M flow by about 20% as compared to simul-…sly injected 10 μm Ms in the dog kidney …n et al. 1979 c). Similar results have been …ed in rats by Yarger et al. (1978), Mimran & … (1979) and Bankir et al. (1979). These find-…dicate that the present absolute Ms to DI …ty in controls might overestimate real zonal …ular to postglomerular flow differences. …ver, neither these recent findings nor other …iprove the concept that 15 μm Ms truely … relative changes of glomerular flow within … one.

…ummary, it should be emphasized that both 15 … and DI indicated that vascular conductance …h of the zones increased and decreased during …ilation and vasoconstriction respectively. …er, whereas flow in all renal zones re-…ed in proportion to total RBF with DI, this …e case for C_1 only with Ms. On average Ms …timated C_1 flow by about 20% and under-…ated C_3M flow by nearly 40% in control kid-…as compared to DI. This disparity tended to …se during vasoconstriction, but practically …

disappeared during maximal vasodilation, suggesting a variable net postglomerular cortical flow with radial direction.

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effect of β-receptor blockade on adrenaline-induced ges in redox and energy state in m vastus lateralis m soleus of the rat in vivo

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FELLENIUS E., HEDBERG, R. & KARLSSON N. The effect of β-receptor blockade on adrenaline-induced changes in redox and energy state in m. vastus lateralis and m. soleus of the rat in vivo. Acta Physiol Scand 1980, 110: 259–266. Received 25 Jan 1980. ISSN 0001-6772. Department of Analytical Chemistry and Biochemistry AB Hässle Mölndal and Institute of Zoophysiology University of Uppsala, Sweden.

The metabolic changes in blood red (m. soleus) and white (m. vastus lateralis) skeletal muscle fibres were investigated after short-term (3 min) infusion of adrenaline with or without prior treatment with propranolol or metoprolol. The adrenaline-induced increase in plasma lactate levels was totally prevented by prior treatment with metoprolol or propranolol whilst the β-blockers had no effect on blood glucose levels. Similar effects on lactate levels were found in the m. soleus, while metoprolol was less effective than propranolol in m. vastus lateralis. Adrenaline decreased the level of muscle creatinine phosphate and ADP causing the equilibrium of the creatine kinase reaction to change in the direction of ATP synthesis, although the level of ATP usually decreased. This effect was more pronounced in m. vastus lateralis compared with m. soleus. The [ATP]/[ADP][Pi]-ratio tended to increase during infusion of adrenaline. This effect was counteracted by metoprolol but not by propranolol. The effects on the "phosphate potential" ([ATP]/[ADP][Pi]) and the equilibrium within the creatine kinase were more pronounced in m. vastus lateralis than in m. soleus. The results demonstrate the possible role of receptors other than β-receptors, i.e. α-receptors, in mediating changes in plasma glucose levels, while plasma lactate levels are regulated by the β-adrenergic system. The role of β-receptors in mediating changes in muscle lactate levels may differ in m. soleus and m. vastus lateralis with relative predominance of β_1-receptors in m. vastus lateralis. Quantitative and qualitative differences in the adrenergic control of the energy state in the two types of muscle fibre were obvious, although it was not possible to distinguish clearly between the relative importance of α, β_1 and β_2-receptors.

Key words: Adrenaline, propranolol, metoprolol, redox and energy state, red and white muscles

ral investigators have recently shown that β-ers decrease the time till exhaustion during uous exercise both in man (Banks et al. 1978, ees et al. 1979) and the experimental animal n 1977). Muscle fatigue may be one reason for effect. Other possible explanations are dis-ed electrolyte balance (Carlsson et al. 1978) or ced substrate supply resulting from decreased and muscle glycogenolysis and adipose tissue ysis (Galbo et al. 1977, Fellenius et al. 1979). kutz (1978) found that glucose infusion restores animals' ability to resume exercise during pro-olol treatment.

In order to gain more insight into the possible role of β-adrenergic control in muscle metabolism, the redox and energy state and substrate levels were measured in a glycolytic (superficial portion of m. vastus lateralis) and an oxidative (m. soleus) muscle of the anesthetized rat during β-receptor activation and inhibition. The animals were exposed to the agonist adrenaline for a very short time (3 min). This design is of special relevance in view of the finding that the β-adrenergic system rather than the energy demand appears to play the cardinal role in the mobilization of energy sources during exercise (Issekutz 1978).

Table 1 *The effect of adrenaline propranolol and metoprolol on blood levels of glucose la... pyruvate* (μmol/g wet wt)

The results are means ± S D with the number of observations in parentheses. Details of the experimental... given in Materials and Methods

Experimental conditions	Glucose	Lactate	Pyruvate	Lactate pyruv...
Control T=0 min	6 04±0 41 (7)	1 44±0 1 (7)	0 1 4_0 018 (5)	11 9+9 6 (5)
Saline + saline	4 96±0_1 (7)	0 99±0 70 (7)	0.075_0 01 (7)	13.1+1 9 (7)
Saline + adrenaline	6 4 ±0.89 (11)	1.88±0 77 (8)	0 139±0 025 (7)	13.8 0 (7)
Propranolol + saline	5 61±0 67 (6)	0 97±0 09 (6)	0 096±0 007 (6)	9 7 1 1 (6)
Propranolol + adrenaline	6.89±0 86 (8)	0 93±0 07 (7)	0 070±0 010 (7)	13 5± 3 (7)
Metoprolol + saline	5 59_0.30 (6)	0 70±0 11 (6)	0 055±0 018 (6)	13 4 3 (6)
Metoprolol + adrenaline	6.35±0.28 (9)	1 03±0 10 (8)	0 074±0 013 (7)	14 1 1 (7)
Statistically significant difference (P<0 05)				
S+S vs. S+A	×	×	×	
P+S	×			
P+A	×		×	×
M+S	×	×		
M+A	×			
S+A vs. P+A		×	×	
M+A		×	×	
P+S vs. P+A	×		×	×
M+S		×	×	×
P+A vs M+A				
M+S vs M+A	×	×		

Table 2 *The effect of adrenaline propranolol and metoprolol on tissue metabolite levels of ... lateralis* (μmol/g wet wt)

The results are means ± S D The number of observations is 5 unless otherwise stated in parentheses

Experimental condition	ATP	ADP	AMP	P	CP
Control T=0 min	5 77±0 36 (4)	0 663±0.073 (4)	0 0_5±0 006 (4)	13 3±3 4 (4)	13 3+...
Saline + saline	5 61±0 64	0.849±0 049	0 03_±0 019	13 7±3 1	[illegible]
Saline + adrenaline	5 03±0.44 (7)	0 663±0 110 (8)	0 031±0 018	10 8_0.4 (6)	[illegible]
Saline + propranolol	5.38±0.46	0 707±0 028	0 0_3±0 007	15.5±3 7	[illegible]
Adrenaline + propranolol	4 49±0.60	0 60_±0 07?	0 026±0 004	9 0_1 6	[illegible]
Metoprolol + saline	6.22±0 47	0.875±0 085 (7)	0.0 1±0 014	19 8±1 6	[illegible]
Metoprolol + adrenaline	4 04±0 4	0 620±0 05_	0 029±0.007	1 6±1.5	[illegible]
Statistically significant difference (P<0 05)					
S+S vs. S+A		×			×
P+S		×			
P+A		×			
M+S					×
M+A	×	×		×	×
S+A vs P+A				×	
M+A	×	×		×	
P+S vs. P+A		×			×
M+A		×		×	×
P+A vs. M+A				×	
M+S vs. M+A	×	×		×	

RIALS AND METHODS

Sprague-Dawley rats weighing 200–230 g, pur rom Anticimex, Sollentuna, S eden, were used. were fed ad libitum on a standard small animal os, Sodertälje, S eden) and ere allowed ess to water prior to the expts.

d analytical grade laboratory reagents were obmed E. Merck AG, Darmstadt, West Germany s and coenzymes ere obtained from Boehringer Mannheim, West Germany or Sigma Chemi- St Louis, MO USA. Solutions of hexobarbital (inh) ere freshly prepared from Evipan -sodium AG, Leverkusen, West Germany). Propranolol ®) and metoprolol (Seloken) ere purchased 1, Pharmaceutical Comp Göteborg, S eden and ssle Pharmaceutical Comp Molndal Sweden, re ely

ental procedure

ere anesthetized by an intra-peritoneal injection of rbital (30 mg/kg body t) and mounted on an op- plate. Skin covering the right hind-leg was re- Fat covering m vastus lateralis as removed and soleus as localized by blunt dissection. Saline were used to prevent surface evaporation from the muscle tissue during the experiment. The abdominal cavity was opened with midline incision and loose fat covering the inferior vena cava and the ilio-lumbar vessels was removed. Propranolol (7 0 µmol/kg body wt), metoprolol (6 0 µmol/kg body wt) or saline was slowly injected (0.25 ml over 2 min period T 0 min.) into the right ilio-lumbar vein. At T=25 min cannula, connected to an infusion pump was placed in the right ilio-lumbar vein for the infusion of adrenaline. At T=30 min the infusion pump was started and adrenaline (33 pmol/kg body wt) or saline was infused over 3 min period. The injected compounds were dissolved in 0.9% NaCl and the total olume of each agent injected was 0.2–0 3 ml. Controls were given saline at T 0 min and at T=30 min and are referred to as saline + saline. The influence of the period of recovery on metabolic levels was also investigated by means of samples taken immediately after the dissection (T=0 min). These e periments are referred to as control, T=0 min

	Lactate	Pyruvate
	2 63 0 35 (4)	0 207±0 004 (4)
	3 26+0 82 (7)	0 259+0 034 (6)
	4 60+0 69	0.278±0 046
	1 57±0 7?	0 165±0 031
	2 17 0 47	0 134±0 018
	46+0 40	0 233±0 040
	3 60+0 40	0 18±0 026

x

Sampling of muscle and blood

3 min after the infusion of adrenaline blood sample (2 ml) was drawn from the inferior vena cava into heparinized syringe. Muscles were then rapidly excised from the right hind-leg and frozen with aluminium tongs cooled in liquid nitrogen (Wollenberger et al. 1960). The muscles were: m. soleus ("red") consisting mainly of slow-twitch oxidative fibres (Ariano et al. 1973) and the superficial portion of m. vastus lateralis ("white") consisting mainly of fast-twitch glycolytic fibres (Ariano et al. 1973 Baldwin et al. 1972). In some experiments the corresponding muscles from the left hind-leg were used for the determination of glycogen content.

Analytical methods

Blood samples were added to 0 6 M $HClO_4$. The extracts were neutralized with KOH and the precipitated $KClO_4$ was removed by centrifugation. The concentrations of lactate glucose and pyruvate were measured in these extracts as described previously (Fellenius et al. 1973 Karlsson et al. 1975)

$HClO_4$ extracts of muscle tissue were prepared, neutralized and the metabolites analysed as described previously (Fellenius et al. 1973 Karlsson et al. 1975) Muscle tissue was hydrolyzed in 5 M KOH for the analysis of glycogen as described by Pfleiderer (1962). Student' *t*-test was used for statistical analysis, $P<0.05$ being considered statistically significant

Abbreviations

Adenosine-triphosphate (ATP), adenosine-diphosphate (ADP) adenosine-monophosphate (AMP) inorganic phosphate (P_i), creatinine-phosphat (CP), creatine (C).

RESULTS

Metabolit s i blood

Blood concentrations of glucose lactate and pyruvate are shown in Table 1 A tatistically ignificant difference was found in glucose and lactate levels

Table 3 *The effect of adrenaline pr pranolol and metoprol l on the red v and energ uae n vastus lateralis*

The results are means ± S D of 5 observations unless otherwise stated in parentheses. Details of the difference given in the text

Experimental conditions	[ATP] / [ADP][P]	[ATP][C] / [ADP][CP]	EC	[ADP]² / [ATP][AMP]	[illegible]
Control T=0 min	0 67±0 1 (4)	6 84±0.35 (4)	0 918±0 003 (4)	3 5±0.54 (4)	[illegible]
Saline + saline	0 53±0 4	3.85±0 43	0 918±0 017	1 +0 74	[illegible]
Saline + adrenaline	0 65±0 06 (6)	6.20±1.59 (6)	0 928±0 01	10±0.70	[illegible]
Propranolol + saline	0.51±0 13	4 28±0.5	0 931±0 006	7.66±4.42 (4)	[illegible]
Propranolol + adrenaline	0 85±0 13	4 90±0 48	0 9 7±0 006	3 1±0 86	[illegible]
Metoprolol + saline	0 35±0 04	3.50±0 1	0.927±0 004	8.25±5 13	[illegible]
Metoprolol + adrenaline	0 5 ±0 08	5 16±0 68	0 918±0 005	3.53+1.04	[illegible]
Statistically significant difference ($P<0.05$)					
S+S vs. S+A		×			
P+S				×	
P+A		×			
M+S				×	
M+A		×		×	
S+A vs P+A					
M+A	×			×	
P+S vs. P+A	×				×
M+S	×				
P+A vs M+A	×				
M+S vs M+A	×	×	×		×

Table 4 *The effect of adrenaline propranolol and metoprolol on tissue metabolite levels of the* (μmol/g wet wt)

The results are means ± S D The number of observations is 5 unless otherwise stated in parentheses

Experimental condition	ATP	ADP	AMP	Pi	Cr
Control T=0 min	3 66±0 20 (4)	0 771±0 038 (4)	0 039±0 004 (4)	8.8±0 6 (3)	[illegible]
Saline + saline	4.39±0 48 (6)	0 813±0 107 (6)	0 044±0 008 (4)	11 0±0.8	[illegible]
Saline + adrenaline	3 99±0 35 (6)	0 736±0 079 (6)	0 049±0 011 (6)	8 4±1 9	[illegible]
Propranolol + saline	3 67±0 13	0 717±0 042	0 028±0 02	10 1±0 6	[illegible]
Propranolol + adrenaline	3 40±0 3	0 713±0 066	0 044±0 007	7 8±1 0	[illegible]
Metoprolol + saline	4.3 ±0 04	0.815±0 079	0 026±0 010	10.3±0.8	[illegible]
Metoprolol + adrenaline	3 01±0 19	0 703±0 023	0.065±0 005	10 4±0.4	
Statistically significant difference ($P<0.05$)					
S+S vs S+A					×
P+S	×				×
P+A	×			×	
M+S					×
M+A	×		×		
S+A vs P+A	×				
M+A	×				
P+S vs. P+A				×	×
M+S	×				
P+A vs. M+A	×			×	
M+S vs M+A	×	×	×		×

control, T=0 min and the saline + sa-
ke infusion of adrenaline caused an in-
n blood glucose concentration compared
control An increase in blood glucose com-
ith the control was also seen when pro-
or metoprolol was given 30 min before the
of adrenaline or saline However the in-
n blood glucose after propranolol and
lol alone was lower than that after the infu-
adrenaline, with or without pretreatment
-blocker A statistically significant increase
 lactate was seen after adrenaline infusion
 increase was abolished when a β-blocker
en 30 min before adrenaline As compared
control, a decrease in lactate concentration
en after the administration of metoprolol
s in blood pyruvate levels essentially paral-
e changes in lactate levels.

ate content of m t s lateral

ncentrations of adenine nucleotides creatine
ate creatine lactate and pyruvat in muscle
re shown in Table 2. The recovery period of
tes after dissection had itself an effect on
f the metabolites. Thus, ADP CP lactate
ruvate levels increased in saline–saline

	Lactate	Pyruvate
4)	1 38+0 19 (4)	0 151±0.010 (4)
	1.53±0 33	0 080±0.030 (4)
	2 22±0 45	0 135±0 024
	1 12+0 18	0 144±0 014
	1 22±0 32	0.112±0 025
	0 85±0 31	0 145±0.007
	1 35±0 45 (6)	0 089±0 025

compared to control T=0 min" The infusion of adrenaline caused an increase in lactate levels, while the concentrations of CP and ADP decreased

The adrenaline-induced increase in lactate was abolished by the β-blockers, propranolol being the more efficient. The changes in CP levels induced by the β-blockers alone differed significantly from those induced by β-blockers plus adrenaline Similar effects were observed on ADP levels ATP levels were relatively constant in the experiment with metoprolol + adrenaline where a decrease was observed compared to metoprolol + saline" or saline + saline

The products [ADP]²/[ATP] [AMP] and [ATP] [C]/[ADP] [CP] which reflect the equilibrium states of the adenylate kinase and creatine kinase reaction respectively are shown in Table 3 together with the cytosolic redox state ([lactate]/[pyruvate] ratio) the phosphate potential" ([ATP]/[ADP] [Pi]) and the energy charge (EC), which reflects the ratio [ATP+1/2 ADP]/[ATP+ADP+AMP] (Atkinson 1968) The [lactate]/[pyruvate] ratio showed only small changes The ratio was about the same in the presence of saline + adrenaline propranolol + adrenaline and metoprolol + adrenaline In the experiments with β-blockers alone the ratio tended to decrease compared with those in which both β-blockers and adrenaline were used Both propranolol and metoprolol tended to increase the ratio [ADP]²/[ATP] [AMP] The creatine kinase reaction moved in the direction of ATP synthesis during adrenaline infusion and this effect was not counteracted by the β-blockers. The phosphate potential was little affected by adrenaline infusion unless the animal had been treated with a β-blocker

Metabol te cont nt of m soleus

In general very much smaller quantitative changes in metabolite levels during β-receptor activation or inhibition occurred in the m soleus (Table 4) than in the m. vastus lateralis. During the recovery phase, i.e control T=0 min versus the saline + saline experiment, ATP P and CP increased statistically significant ($P<0.05$). Adrenaline infusion had very little effect on metabolite levels. In the presence of metoprolol, adrenaline induced a relatively pronounced drop in the level of ATP while no corresponding significant changes occurred with propranolol Propranolol alone tended to decrease ATP CP lactate and pyruvate levels

Table 5 *The effect of adrenaline propranolol and metoprolol on the redox and energy state of m soleus*

The results are means ± S D of 5 observations unless otherwise stated in parentheses Details of the conditions are given in Materials and Methods

Experimental conditions	[ATP] / [ADP][P]	[ATP][C] / [ADP][CP]	EC	[ADP]² / [ATP][AMP]	[Lac] / [Pyr]
Control T=0 min	0 53±0 07 (3)	4 63±0 79 (4)	0 891±0 005 (4)	4 34+0 40 (4)	[illegible]
Saline + saline	0 48±0 04	4 59±0.33 (4)	0.898±0 003 (4)	[illegible]76+0 94	[illegible]
Saline + adrenaline	0 65±0 [illegible] (6)	5.37±0 70	0.900±0 01 (6)	1 86±0 43	[illegible]
Propranolol + saline	0 49±0 05	4 7[illegible]±0.34	0 899±0 011	4 99+[illegible]27 (6)	[illegible]
Propranolol + adrenaline	0 6[illegible]±0 11	4.29±0.[illegible]4	0 890±0.006	3 46+0 64	[illegible]
Metoprolol + saline	0 53±0 08	4 73±0 64	0 903±0 006	5 76± 96	[illegible]
Metoprolol + adrenaline	0 41±0 0	4 04±0 4[illegible]	0 873±0 006	.55±0 13	[illegible]
Statistically significant difference (P<0 05)					
S+S vs S+A					x
P+S					
P+A	x		x		x
M+S					
M+A	x		x		
S+A vs P+A		x		x	x
M+A	x	x	x		
P+S vs P+A	x	x	x		x
M+S					
P+A vs M+A	x		x	x	x
M+S vs M+A	x		x		

while metoprolol alone produced statistically significant effects only in lactate and pyruvate levels

Effects on the [lactate]/[pyruvate] ratio and the [CP] ratio (Table 5) were found during the recovery phase i e control T=0 min versus "saline saline Adrenaline only affected the energy and redox state in the presence of a β-blocker Thus the phosphate potential was increased by adrenaline after prior treatment with propranolol while the reverse occurred with metoprolol As compared to the control both β blockers produced a significant lowering of the [lactate]/[pyruvate] ratio Adrenaline increased the [lactate]/[pyruvate] ratio after prior β receptor blocker treatment

Effect on glycogen content

Glycogen levels (Table 6) changed very little during the recovery period although a statistically significant decrease in glycogen was observed in m soleus There were no statistically significant effects on glycogen stores during adrenaline infusion The effect of adrenaline during β-receptor blockade was complex After propranolol treatment adrenaline induced an increase in glycogen stores in soleus but not in vastus lateralis. After [illegible] pretreatment adrenaline caused no effects [illegible] but a slight decrease in glycogen stores was [illegible] times found in vastus lateralis

DISCUSSION

The dose of adrenaline was chosen to [illegible] stimulation in heart rate caused by a maximal [illegible] of the β agonist isoprenaline (Fellenius [illegible] lished observation) The adrenaline induced [illegible] crease in plasma glucose levels was not [illegible] by prior β-receptor blockade This is in [illegible] with the possible mediation of hepatic glucose [illegible] lease by α receptors suggested by Ellis (1979)

The adrenaline induced increase in plasma [illegible] tate levels was reversed by both propranolol [illegible] metoprolol (Table 1) Similar effects were [illegible] m soleus (Table 4) while metoprolol was less [illegible] fective than propranolol in m vastus lateralis [illegible] ble 2) This would indicate that the role of β-[illegible] tors in determining muscle lactate level may [illegible] in m soleus and m vastus lateralis with a [illegible] predominance of β_1-receptors in the m [illegible]

The effect of adrenaline, propranolol and ...ol on glycogen content in m. vastus ...-and m. soleus

...s are means ± S.D. of 5 observations unless stated in parentheses. For details of experimen... see Materials and Methods

	Glycogen (μmol glucose/g wet wt)	
...ental conditions	Vastus lateralis	Soleus
T=0 min	36 +1 9	23 0±2 5
saline	36 6±8 3 (8)	19 7±1 4
adrenaline	24.7 1.9	17.6±2 1
...olol saline	31.8 4 6	17 5±2.3 (4)
...olol adrenaline	33 3 3 2	25 9±2.5
...lol saline	40 4 5	4.0±2 0
...lol adrenaline	31 8±8 7	25 9±0 5

...ally significant difference ($P<0.05$)

S+A
P S
P A
M S
M A

P A
M A

P A
M S

M A

M A

... A β_1-receptor influence in the soleus mus... ...ther surprising in view of previous findings ... relative importance of β_2-receptor in muscle ...nolysis (Arnold 1972, Stanton 1972, Kuo et ...7, Hestmark & Horn 1975). However previ... ...dies did not differentiate between slow and ...itch fibres. Furthermore the possibility of a ...nsatory mechanism secondary to β-blockade ...e considered before the type and population ...ceptors can be stated. Thus, any decrease in ... glycogenolysis and consequently in lactate ...tion may be compensated for by an increase ...cose uptake resulting from activation of ...nase by decrease in glucose-6-phosphate. This would restore lactate production and ...ly also the level of lactate. The fact that ... release is unaffected by β_1-receptor block... ...eatment (William-Olson et al. 1979) in con... ...o propranolol treatment, may in addition ...ract the adrenaline induced increase in mus... ...cogenolysis.

Adrenaline may also have some α-adrenergic effects. α-receptors may mediate an effect opposite to the β-effect on muscle metabolism (Dynarowicz 1974 *a*, *b*).

Besides a direct adrenergic control of skeletal muscle metabolism, an indirect control in the form of adrenergic regulation of skeletal muscle blood flow may exist. This would affect the rate of del... very of substrate which is essential for the maintenance of normal energy and redox states. It is well documented that the two β-blockers may influence the peripheral resistance differently (Åblad et al. 1975).

Although it is not possible to evaluate the relative importance of α, β and β_2-receptors within the different muscle groups, both quantitative and qualitative differences in adrenergic control seem to exist in the two muscle fibres.

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...s of short term and prolonged infusions of ...tostatin on endocrine pancreas body weight ...od intake in rats

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LINS P E., PETERSSON B & EFENDIĆ S Effects of short-term and prolonged infusions of somatostatin on endocrine pancrea body weight and food intake in rats Acta Physiol Scand 1980 110 267-275 Received 25 Jan 1980 ISSN 0001-6772. Department of Endocrinology Karolinska Hospital Stockholm and Department of Histology University of Uppsala, Sweden

Infusion of cyclic somatostatin (700 ng/kg/min) for 4 h in rat fed ad libitum suppressed basal insulin but not glucagon release. It was accompanied by hypoglycemia during the first hour whereas, at the end of the infusion hyperglycemia was present The same dose of somatostatin applied 60 min prior to and during a 30 min load of glucose or arginine significantly inhibited their effects on insulin and glucagon release. In contrast, when this dose of somatostatin was given during 24 h period by the i route it did not inhibit glucose induced insulin release though circulating somatostatin levels were constantly and markedly elevated. Furthermore in rats continuously infused with somatostatin for 4 days, no effect was found either on plasma concentrations of glucose, insulin, glucagon growth hormone and cyclic AMP or on body weight gain, food consumption or water intake The pancreases of these animals showed normal concentrations of insulin and glucagon and normal nuclear area of D-cells. Our experiments demonstrate that, in short-term experiments in rats, somatostatin influences insulin and glucagon release as well as glucose homeostasis Furthermore they suggest that during prolonged administration of somatostatin rats develop mechanisms counteracting the effect of the peptide e.g peripheral tachyphylaxis.

Key ords Somatostatin endocrine pancreas

...statin in short-term experiments in man and ... inhibits the release of growth hormone ...sulin glucagon and several gastrointestinal ...es (Efendić et al. 1978) It also decreases ...stinal absorption of xylose (Felig & Wahren ...nd reduces food intake (Lotter & Woods ... This would imply that prolonged adminis... of somatostatin might interfere with blood ... homeostasis and in addition, inhibit growth ...sorption of nutrients from the gastrointestinal ... For this reason we have investigated the ... of short-term and prolonged administra... somatostatin in rats on the functions of the ...ne pancreas, as well as the influence of ...ged administration (4–14 days) of the peptide ...dy weight and food intake

MATERIAL AND METHODS

Animals. Male Sprague Dawley rats weighing approximately 200 g were used throughout the expts. The animals were kept on complete pellet diet (Ewos Standard Astra Ewos, Södertälje) and water ad libitum. In the expts. dealing with the effect of somatostatin on glucose and arginine stimulated insulin and glucagon release food was withdrawn at 2 p m. on the day preceding the expt. In the studies on the effect of the peptide on basal secretion of pancreatic hormones, the rats had free access to pellet food and to tap water

Surgical procedure Short-term infusions of glucose and arginine with or without somatostatin were given under penthotal anesthesia (sodium penthotal 12.5 mg/100 g of body weight intraperitoneally). T allow blood sampling during the infusions, both external jugular veins were cannulated and short teflon catheters inserted. Blood was collected into chilled heparinized tubes. Plasma was separated and stored until assayed

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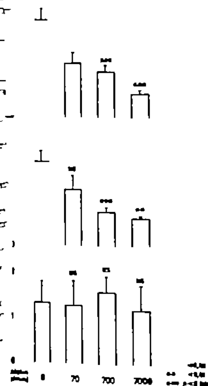
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ffect of somatostatin on insulin, glucagon and responses to arginine. Somatostatin was given 60 ... and during 30 min arginine infusion (40 ...). The responses are calculated as the dif between values obtained at the end and at the ... arginine infusion (mean ± S E)

tions by means of an ocular screw micrometer ...tion 1250). The largest nuclear diameter was ... as well as the diameter at right angles to it. ...th their centres within the sections were ... The areas of the largest optical cross sections ... were then calculated in arbitrary units

Synthetic cyclic somatostatin was obtained from ...rch Department of the Kabi Group, Stockholm ... gift from AB Vitrum, Stockholm.

TS

of 90 min infusions of somatostatin on induced insulin release and arginine insulin and glucagon release in fasted rat ... also increment during a glucose load (20 ...) was significantly reduced by somato-... dose of 700 ng but not by 70 ng/kg/min

(Fig. 1). The type of interaction of glucose and somatostatin on insulin release was analysed by combining the somatostatin infusion (700 ng/kg/min) with increasing i.v. loads of glucose (5 10 20, 30 60 and 100 mg/kg/min) The insulin response to glucose alone increased with the dose of the hexose but even at such a high glucose load as 100 mg/kg/min. Maximal insulin response was not achieved (Fig. 2). Somatostatin significantly inhibited the insulin response to all glucose doses applied and this effect could not be overcome by the levels of hyperglycemia attained. Therefore in these experiments the interaction of glucose and somatostatin seemed to be non-competitive

Arginine (40 mg/kg/min) enhanced insulin and glucagon secretion (Fig. 3). Somatostatin at a dose of 70 ng/g/min, significantly inhibited insulin release A higher dose of the peptide (700 ng/kg/min) was required for inhibition of glucagon release The hyperglycemic response to arginine was not modified by somatostatin

Effects of a 4 h infusion of somat statin on basal lev ls of glucose insulin and glucagon in fed rats In control animals given saline alone the blood glucose concentrations decreased during the whole expt. (Fig. 4). The lowest glucose levels were observed after 2 h In the presence of somatostatin (700 ng/kg/min) the blood glucose pattern was altered: after 1 h more marked hypoglycemia was noted after 2 h there was no change as compared to the controls and finally at 4 h significantly higher blood glucose levels were recorded Insulin secretion was suppressed during the whole expt. in the somatostatin animals. This inhibition was even more obvious when insulinogenic indices were compared. In contrast, somatostatin only slightly suppressed basal glucagon secretion

Effects of a 24 h infusion of somatostatin on glucose induced insulin elease in fasted ats In rats infused over 24 h with saline a 30 min infusion of glucose (30 mg/kg/min) applied at the end of expt. induced a marked insulin release (Fig 5). This effect of glucose was significantly inhibited by somatostatin (700 ng/kg/min) given 60 min prior to and during glucose infusion I contrast, somatostatin had no significant effect in animals that received the peptide during the whole experiment.

Effects of infusions of somatostatin for 4–14 days on plasma levels of glucose insulin glucagon cy lic AMP and GH on insulin and glucagon content and the number f D-cells in the pancreas as well

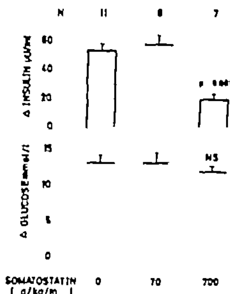


Fig 1 Effect of somatostatin on insulin and glucose re sponses to glucose Somatostatin was given 60 min prior to and during a 30 min infusion of glucose (?0 mg/kg/min) The responses were calculated a the differences between values obtained at the end and at the tart of glucose infusion (mean ± S E)

In the long term expts the rats were operated one week before the start of the admini tration of somat statin At this operation the superior vena cava was cannulated from the external jugular vein The proximal end of the catheter was brought out through the kin in the midscapular area and held in a protecting harness (Weeks 1971) The catheter was then drawn through a protecting tube connected to a ball-bearing system in the roof of the metabolic cage This allowed the animal to move freely without damaging the infusion system

In the 4 h expt food was withdrawn at p m on the day preceding the expt One group of rats received saline and another somatostatin (700 ng/kg/min) over 4 h A third group of animals was infused with saline f r ..5 h and thereafter received a 90 min infusion of somatostatin All animals were anesthetized with penthotal after ..5 h and the other external jugular vein was catheterized for application of glucose (30 mg/kg/min) during the last 30 min of expt The same stock solution of somatostatin wa prepared and kept at room temperature for 24 h and 90 min infusions.

In the 4 and 14 days expts, somatostatin or saline alone were administered as continuous infusions except for a morning break of one hour During this break the rats were weighed and the consumed f od and water was measured At the end of expt in the non-f sted tate the rats were killed by decapitation at 8 a m and blood was ollected for determination of glucose insulin glucagon GH and cyclic AMP The somatostatin infusion was con tinued during decapitation and blood sampling A piece of the pancreas was immediately dissected and frozen in liquid nitrogen while another piece w immersed in Zenker-formalin solution dehydrated and embedded in paraffin

Extraction of tissue and hormone assays Th pieces of the pancreas were sonicated in appr volumes of cold acid ethanol (0 18 M HCl a ? e and extracted for 16–18 h at 4°C After cent 000×g for 20 min at 4°C the supernatant wa and stored at −20°C until assayed The sol ext tract was d luted with the buffer used for ra say s Insulin was determined by a double-antibod immunoassay (Hales & Randle 1963), glucagon b coal separation (radioimmunoassay using ... (Falo0na & Unger 1974), GH by a radioim technique (Edén et al 1978) and cyclic AMP by kinase binding assay (Gilman 1970) Glucose was measured by a glucose oxidase method (L Nixon 1957) Somatostatin was measured b munoassay (Efendic et al 1978) The antibo duced by oursel es were used at a final c 1 56 000 The antigenic specificity of the antibo determined using somatostatin analogs (Ver 1978) Crossreactivity of the antibody as le with insulin glucagon substance P LH RH ... and oxytocin Somatostatin in plasma was mea acetone extraction (Arimura et al 1978) ... index was calculated as the ratio between pro sulin glucose concentrations.

Quantitative hist logy of the islets Paraffin from the Zenker formol fixed pancreas we impregnated (Hellerström & Hellman 1960) Th were photographed and the sections restained morf's chrome hematoxylin ponceau fuchsin Th (A₁-cells) were identified by comparing the phot with the granule stained sections (Petersson et al The nuclear size of the cells was determined b metry In each animal 4 D-cells were measured

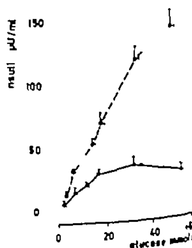
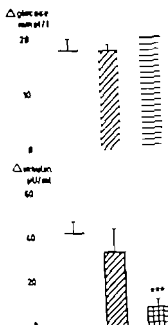


Fig 2 Interaction of somatostatin and glucose on release Somatostatin (700 ng/kg/min) as given prio to and during a 30 min infusion of glucose (30 60 and 100 mg/kg/min) The responses were cal as the differences between values obtained at the e at the tart of glucose infusions (mean ± S E) circles and solid lines denote experiments with s statin open circles and broken lines denote cont periment



ffect of somatostatin (700 ng/kg/min) on glucose in responses to 30 min infusion of glucose (30 n) in fasted rats. One group of rats received yes bars), and another somatostatin (stretched 4 h. A third group of animals (checkered bars) saline for 22 5 h and somatostatin for 90 min. The ere calculated as the differences between val med at the end and at the tart of glucose infusion S E.) *** P<0 001. For details see Methods.

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esent series of e pts have demonstrated the rat, the duration of somatostatin stration is of significance for its action on the pancreatic functions. In general the in- y effects of the peptide on insulin and

1 *Plasma levels of gluco insulin on, growth hormone nd cyclic AMP (mean) 4 day afte th start f th somatostatin* n

	Somatostatin (11)	Controls (=11)
se mmol/l	7.3± 0 4	6 9± 0.1
n, μU/ml	77 8± 1 7	27 8± 2 6
gon, pg/ml	338 0±54 0	394 0±73.0
h hormone ng/ml	196 0±97.0	127.5±56.7
pmol/ml	45 6± 1.8	43 6_ 1.8

Table 2. *Insulin and glucagon levels in extracts from pancreas (mean ± S.E) from rats sacrificed 4 days after the start of the somatostatin infusion*

	Somatostatin (=11)	Controls (=11)
Insulin μU/mg wet weight	1 517.0±199.0	1 429 0_215.0
Glucagon ng/mg wet weight	2.9± 0 6	_3± 0 4

glucagon release present after 4 h of infusion seemed to disappear when the administration was extended over 4 h

Somatostatin (700 ng/kg/min) was a strong in- hibitor of basal insulin release in the present short term expts We have previously demonstrated that, in the rat, this dose of the peptide exerted a maximal inhibitory action on basal insulin release (Efendić et al. 1978) In the present study it was demonstrated that the same dose of the peptide also inhibited glucose induced insulin release It also in- hibited arginine induced insulin and glucagon re lease which confirms previous observations (Gor din et al 1977 Brown et al 1976) However basal glucagon release was not suppressed by this dose of the peptide which also is in agreement with the observations of others (Dunbar et al 1978)

Somatostatin, under prevailing in vivo condi- tions did not inhibit basal glucagon release Since it strongly inhibited basal insulin an o erall hyperglycemic effect could be expected This was not the case during the first hour of infusion when somatostatin exerted a marked hyperglycemic ef fect. Most likely this was due to interference of the peptide with the intestinal absorption of nutrients. In this context, it is of interest that, in humans the

Table 3 *Nuclear rea of D-cells (mean ± S.E) in pancreas from rats acrificed 4 and 14 days afte the start of the omato t tin infusio*

Days of infusion	Somatostatin	Controls
4	0.5763±0 0119 (=9	0 5958 0.1390 (9)
14	0.5814±0 0482 (=2)	0.5865±0.0244 (3)

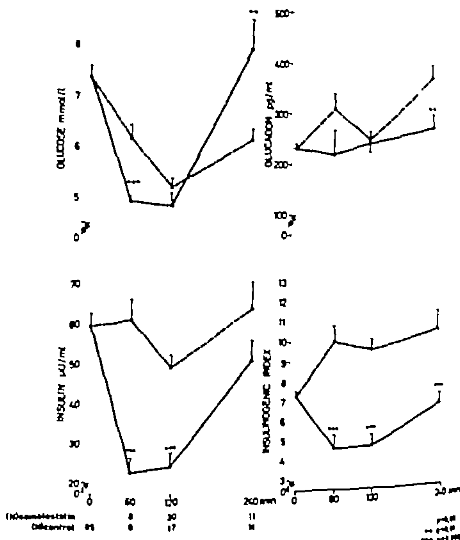


Fig 4 Effect of a 240 min intravenous infusion of somatostatin (700 ng/kg/min) on glucose insulin glucagon and insulinogenic index in fed rats The animals were killed by decapitation at 0 60 120 and 240 min (mean ± S.E) Filled circles and solid lines denote experiments with somatostatin, open circles and broken lines denote control experiments

as on body weight and food consumption Continuous infusion of somatostatin (700 ng/kg/min) for 96 h did not alter the levels of glycemia or of those of the above hormones or cyclic AMP in rats fed at libitum (Table 1) No glucosuria was observed at daily checking of the urine The concentrations of insulin and glucagon in pancreatic extracts were almost identical in somatostatin treated and control rats after 4 days of infusion (Table 2) Likewise quantitative histological studies of the islets of Langerhans did not reveal any effects of 4–14 days infusion of somatostatin on the areas of D-cells (Table 3)

Continuous infusion of somatostatin over 4 days neither influenced body weight nor consumption of food and water (Fig 6) The decrease in ... during the first day of the experiment ... was due to the withdrawal of food on the ... Moreover somatostatin infused for as long ... days did not influence body weight or ... water intake (Fig 7) In these expts new ... statin infusions were prepared every 4 h

Plasma levels of somatostatin in rats ... ly infused with somatostatin Under basal conditions somatostatin levels in peripheral ... ied between 0 and 70 pg/ml In animals infused ... somatostatin (700 ng/kg/min) already 10 ... the start of infusion plasma levels of the ... reached levels of approximately 500 pg/ml ... mained almost ... h (Fig 8)

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explanation is supported by the observation that in anesthetized dogs the suppressive effect of somatostatin on insulin release could be counteracted by the alpha adrenergic blocker phentolamine (Smith et al 1976) In this context it is of interest that in perifused rat islets epinephrine inhibited glucose induced insulin release in a non competitive manner (Campfield 1975)

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ts of blood pressure manipulations on shivering nogenesis in the pigeon

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HOHTOLA, E. SAARELA S. & HISSA, R Effects of blood pressure manipulations on shivering thermogenesis in the pigeon Acta Physiol Scand 1980. 110 277–283 Received 5 February 1980. ISSN 0001-6772. Department of Zoology Zoophysiological Laboratory University of Oulu Finland.

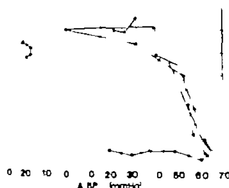
The effects of blood pressure (BP) manipulations on shivering thermogenesis were studied in conscious pigeons. A rise in BP induced by noradrenaline (NA) or equipotent doses of angiotensin II (Ang II) effectively suppressed shivering at +12°C and partly abolished the cold induced vasoconstriction in the feet The inhibition commenced when the rise in BP reached +40 mmHg, and fall in body temperature followed these responses. Comparison of the trajectories in the BP-shivering plane revealed that the inhibition of shivering by Ang II could be completely explained by changes in BP whereas NA had also another more prolonged inhibitory action independent of baroreceptor activity A similar dose-dependence for effects on BP and shivering could be established with both drugs. An acute reduction of BP by sodium nitroprusside had also very potent inhibitory effect on shivering I hypotensive pigeons elevation of BP with NA initially enhanced shivering, but when BP was raised beyond normal levels shivering was again suppressed. We conclude that both rise and fall in BP can inhibit shivering in the pigeon and that normal levels of BP facilitate shivering. NA inhibits shivering by more than one mechanism, but the initial effect is mediated through a baroreflexive action The interactions of thermoregulatory and cardiovascular mechanisms suggest an integrated control of body temperature and circulation, which should be considered in experimental approaches to these homeostatic systems.

Key words Blood pressure, shivering, baroreflexes, temperature regulation, noradrenaline angiotensin II, sodium nitroprusside, pigeon.

erature regulation is usually defined as a ostatic system responding to changes in ther nputs reaching the controlling center Several ns have however hown that a host of ological variables other than temperature af he effector functions of temperature regulation g Hammel 1968) These non-thermal inputs de cardiovascular variables, states of arousal formal factors, for example They have some s been considered in models of body temture control (e.g. Hammel 1968 Gale 1973), but ematic studies on the interaction of temperature dation th other homeostatic functions are few umber

n this study we analyze the effects of blood ssure (BP) manipulations on temperature reg ion in the pigeon These experiments were mpted by the observation that peripherally adnistrated noradrenaline (NA), known to stimulate thermogenesis in new-born and cold-acclimated mammals (see Jansky 1973) suppresses cold-induced shivering in the pigeon (Hissa et al. 1975b) and other birds (Allen et al. 1970) resulting in decreased heat production and lowered body temperature even in the cold-acclimated bird (Hissa et al 1975). The profound cardiovascular effects of this drug and some earlier mammalian studies (viz. Mott 1963 Wasserstrum & Herd 1977) led us to suspect a baroreflexive origin for this inhibitory action This contention was strengthened by the finding that the inhibition could be abolished with prior alpha-receptor blockade (Hissa et al 1975b) which should reduce the pressor effects of peripheral NA

Besides NA we used also angiotensin II (Ang II) more specific vasopressor agent, to induce elevations in BP For lowering the BP sodium nitroprusside (SNP) a potent vasodepressor acting



Trajectories of the changes in blood pressure (BP) vering induced by injections of noradrenaline (solid fied circles) and angiotensin II (broken line open) The arrows show the direction of time beginning he moment of injection The time interval between ccessive circles is 1 min

mpler was recorded without smoothing with VE22 polygraph (Schwarzer)

ering was measured as the EMG-activity of the al muscle using an EMG-processing apparatus con- ed in our laboratory The EMG was picked up by monopolar stainless steel electrodes fixed triangular epoxy resin plate The electrode length was 5 mm e sides of triangle measured 3 mm After insertion, ectrodes ere connected to differential amplifier band-pass filter (the low and high cut-off frequencies d 500 Hz, respectively) The relatively close ar ment of the electrodes attenuated cardiac potentials signal to noise level and spurious potentials arising respiratory movements (<1 Hz) ere eliminated by band-pass filter The amplified and filtered EMG-sig was then full-wave rectified and veraged with an tegrator (time-constant 10) The averaged potential senting shivering intensity as recorded ith the Gould DP-6 potentiometer along with the mean ar l pressure The direct EMG was continuously tored ith Tektronix 520A oscilloscope to ascertain true shivering potentials ere obtained.

dy temperature (T_b) from the cloaca and the tem- ture of the naked tarsometatarsus (T_f) were recorded small diode probes and digital thermometer con- cted in our laboratory Temperatures were plotted on vetter Clearspan P250L-recorder

cedure

vicious pigeons were used The experiments ere be by weighing the bird 3 h after lights-on (6 a.m.) The ron as immobilized ith hood (Evered & Fitzsimons 7) and tethered on the operating table in supine ition ith the wings extended After exposing the od vessels, the BP-cannula and injection cannulae were erted Routinely NA was injected into the tibial vein d Ang II as given intramuscularly into the pectoral scle SNP as infused into the brachial vein. For injec ns the cannulae ere connected to remote syringes

The temperature probes and EMG-electrodes were fixed with adhesive tape and the bird was then returned to an upright position and placed into plexiglas restraining frame The frame allowed the bird to attain normal postures of the wings head and body The legs were tethered and bent towards the tail The bird was then transferred into thermostatically controlled metabolic chamber maintained at +17°C. This temperature elicited strong shivering in the restrained bird (peak-to-peak amplitudes of the EMG 800–1200 μV mean rectified potential approximately 60 μV) The hood was removed the chamber darkened and 0.5–1 h equilibration time was allowed before starting the measurements.

RESULTS

Fig 1 shows the mean effects of BP elevations induced by NA (500 μg/kg i. left panel) and Ang II (50 μg/kg i.m. right panel) The rise in BP after NA-injection is followed by an inhibition of shivering, tachycardia, a fall in T_b, and a partial abolition of the cold-induced vasoconstriction in the feet The rise in BP induced by an equipotent dose of Ang II likewise inhibited shivering, lowered T_b, and elicited foot vasodilatation. The response in HR was clearly biphasic

After the responses had decayed, the level of BP settled 21 (±9.8 S.E.) mmHg below preinjection level ($P<0.05$ paired *t*-test) after NA, whereas after Ang II it settled 23 (±3.9) mmHg above control level ($P<0.002$, paired *t*-test). These shifts did not affect subsequent levels of shivering.

Although shivering was similarly suppressed by the elevation of BP with both drugs marked difference was noted. While shivering returned to normal levels concomitantly with restoration of normal BP after Ang II injection, it was suppressed for a much longer time after NA despite the re

Table 1 *Regression coefficients and their standard deviations relating the change in shivering to changes in blood pressure (BP) between +40 and +65 mmHg after injections of noradrenaline (NA) and angiotensin II (Ang II)*

The coefficient marked by an asterisk is significantly ($P<0.001$) different from all other coefficients. Each coefficient is based on data from 6 pigeons

Phase of BP	Drug NA	Ang II
Rising	−1.99+0.28	−.29±0.11
Falling	−0.18+0.05	−1.90±0.30

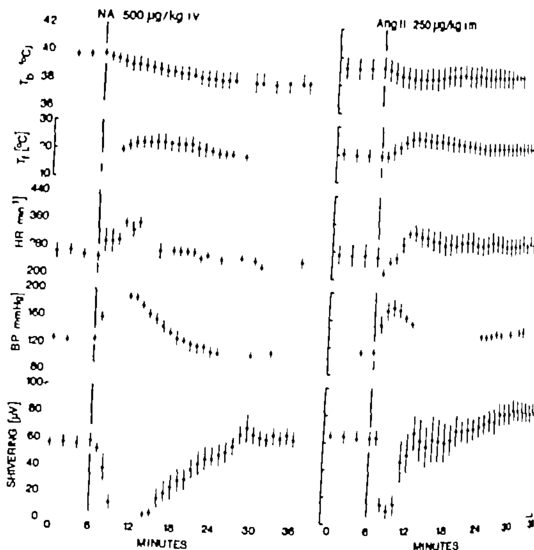


Fig 1 Effects of noradrenaline (NA left panel) and angiotensin II (Ang II right panel) on shivering, blood pressure (BP) heart rate (HR) foot temperature (T) and body temperature (T) in the pigeon at an ambient temperature of ... N = 6 in both groups The maximal changes are all significant ($P<0.05$ or better paired *t* test)

directly on vascular smooth muscle (e g Kreye 1978) was used By manipulating BP with these drugs clear-cut and reproducible changes in shivering could be elicited Preliminary results of these experiments have been presented elsewhere (Hohtola & Saarela 1979)

MATERIAL AND METHODS

Animals

Adult pigeons (*Columba li ia* mass 0.28–0.38 kg) of both sexes were used They were housed in metal cage at +22°C in an animal room with a 1...1 L D light-dark cycle and 40–50% R H for several week prior to experiment Food and water were available ad libitum

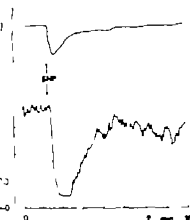
Drugs

Noradrenaline (NA L Arterenol bitartrate Sigma Chemical Company) was dissolved in 0 85% N Cl Angiotensin II (Ang II Sigma Chemical Company) was dissolved in buffered (pH 9) saline Sodium nitroprusside (SNP Sigma Chemical Company) was likewise dissolved in saline All olutions used for intravenous ... were heparinized (approx 100 U I./ml) The concentrations were adjusted to give injection volumes of ... µl

Mea urem nts

Blood pres ure (BP) was measured from the right ... artery A tapering polyethylene cannula (PE ...) with heparinized saline was introduced into the ... near the proximal end of the humerus and connected ... MSD10-S (Alltech) pressure transducer A ... (Schw rzer) strain gauge coupler was used to ... the transducer signal The output of the coupler was fed ... RC-integrator (time-constant 10 s) and the mean ... pres ure thus obtained was recorded with a ... DP-6 (Tohshin) potentiometer All BP values in the present study refer to mean arterial pressure

For measuring the ... a parallel ...



The effects of sodium nitroprusside (SNP 100 ... blood pressure (BP) and shivering in ... representative pigeon at 12°C

...en observed Fig. 5 shows the responses of ... shivering to NA in a representative pigeon ...d hypotensive by controlled bleeding. The ... BP is initially followed by an increase in ...ng intensity but the subsequent rise of BP ...l normal levels (i.e. about 120 mmHg) again ...s shivering. In contrast to the effects of acute ...tension elicited by SNP (Fig. 4) shivering was ...ally inhibited by the chronically lowered BP

...USSION

...resent findings show that BP can profoundly ... shivering in the conscious pigeon. Both a rise ...r fall in BP result in a suppression of shivering. ... discussion that follows is based on the ...heses that baroreceptors have a major role in ...nding these effects. However it should be ...d that baroreceptor activity was manipulated ...ectly via BP-changes.

...at the inhibition of shivering by peripherally ...inistrated catecholamines might be mediated ...y through baroreflexive mechanisms has rarely ... considered and so far only in mammals. Was... ...rman & Herd (1977) demonstrated a barore... ...ive depression of cold-induced oxygen con... ...ption in the squirrel monkey and while this ...er was in preparation, Marshall & Stoner (1979) ...rted that shivering in the rat is inhibited when ... is elevated by NA or methoxamine. That ...oreceptor activity in general affects shivering

was suggested by the early studies of Ishii & Ishii (1960*a* 1960*b*) and Mott (1963). The same authors noted especially that a reduction of BP has a potent inhibitory effect on shivering, which has been confirmed in the present study also for birds.

However by comparing the effects of NA and Ang II it was possible for us to demonstrate that intravenous NA and possibly other adrenergic agents exert their inhibitory effect on shivering through two separate mechanisms: An initial baroreflexive suppression and another more long-acting inhibitory mechanism (Fig. 2). Marshall & Stoner (1979) also mention that shivering did not always reappear in pace with restoration of normal BP after NA-injection in the rat, and Hall & Goldstone (1940) suggest that adrenaline inhibits shivering by more than one mechanism in the cat.

It is possible that the later effect is mediated by a direct action of NA on the CNS in the pigeon. Hissa & Rautenberg (1974) showed that centrally applied NA inhibits shivering in the pigeon and Hissa & Pyornila (1977) were able to alleviate the effects of peripheral NA on shivering by prior intrahypothalamic injection of *phentolamine (alpha receptor blockade)*. However chemoreceptor stimulation also inhibits shivering (Good & Sellers 1957 Mott 1963 Schafer & Wünnenberg 1976). Considering the finding that intravenous NA may elevate plasma pCO_2 in the pigeon (Hissa & George 1980) this mechanism cannot yet be excluded, nor can the possible direct effects on skeletal muscle be disregarded (Bowman & Nott 1969).

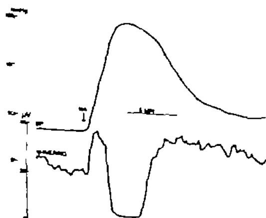


Fig. 5. Effect of NA (300 µg/kg) on blood pressure (BP) and shivering in a pigeon rendered hypotensive by controlled bleeding. Note the initial augmentation of shivering.

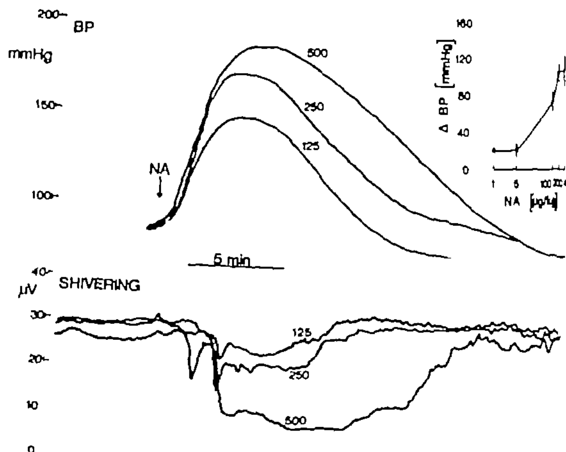


Fig 3 Dose-dependent effects of noradrenaline (NA) on shivering and blood pressure (BP) in the pigeon The N[illegible] shown along the curves are expressed as μg/kg The inset shows the mean effects of NA on BP for a wider range [illegible] that was tested in preliminary expts (N = 5)

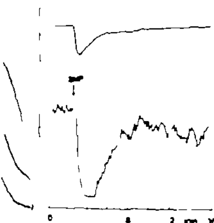
covery of normal BP This correlated with the deeper fall in T_b after NA injection The phenomenon is analyzed in more detail in Fig which shows the trajectories in the BP-shivering plane after injections of NA and Ang II A clear-cut threshold effect is noted Shivering begins to diminish when the increase in BP reaches about 40 mmHg and is completely suppressed after a rise of 65 mmHg The superimposed curves for NA and Ang II coincide almost completely during the rising phase of BP However while the trajectory for Ang II follows an almost identical path also during the falling phase of BP a marked hysteresis in the NA trajectory is observed at this phase i.e shivering remains suppressed despite the normalization of BP In line with this a linear regression analysis relating changes in shivering to changes in BP (between +40 and +65 mmHg) separately for the rising and falling phases of BP indicated that excluding the falling phase of BP after NA-injection the regression coefficients did not significantly differ from each other (Table 1)

The rise in BP needed to inhibit shivering in these experiments was about 40 mmHg The [illegible] showed however a dynamic nature for [illegible] was more abruptly elevated by injection [illegible] brachial vein for example shivering [illegible] mediately suppressed In fact the injection [illegible] were purposively selected to achieve [illegible] rates of increase in BP so that possible [illegible] effects could be evaluated

By injecting NA at various dosages graded [illegible] sponses in BP were obtained These were [illegible] by graded inhibitions in shivering as well (Fig [illegible] The rank order of potency of different NA-do[illegible] was thus the same for changes in BP and shiv[illegible] Ang II also showed a similar dose-dependence [illegible] when injected into the brachial vein elicited [illegible] rises of BP at much lower dose rates (1[illegible] μg [illegible]

An acute reduction of blood pressure with [illegible] (100 μg/kg) had also a very potent inhibitory [illegible] on shivering The abrupt lowering of BP [illegible] immediately followed by cessation of shiv[illegible] (Fig 4)

When NA was injected into chronically hyper[illegible]sive pigeons an initia[illegible] tion of shiver[illegible]



The effects of sodium nitroprusside (SNP 100 ... blood pressure (BP) and shivering in ... representative ... at ... 17°C

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It is possible that the later effect is mediated by a direct action of NA on the CNS in the pigeon Hissa & Rautenberg (1974) showed that centrally applied NA inhibits shivering in the pigeon and Hissa & Pyornilä (1977) were able to alleviate the effects of peripheral NA on shivering by prior intrahypothalamic injection of phentolamine (alpha receptor blockade) However chemoreceptor stimulation also inhibits shivering (Good & Sellers 1957 Mott 1963 Schafer & Wünnenberg 1976) Considering the finding that intravenous NA may elevate plasma pCO_2 in the pigeon (Hissa & George 1980), this mechanism cannot yet be excluded nor can the possible direct effects on skeletal muscle be disregarded (Bowman & Nott 1969).



Fig 5 Effect of NA (500 μg/kg) on blood pressure (BP) and shivering in ... pigeon rendered hypotensive by controlled bleeding Note the initial augmentation of shivering.

Two points strongly suggest that the effects of Ang II unlike those of NA are mediated solely by baroreflexes The first is the lack of hysteresis in the BP shivering trajectory Secondly Görke & Pierau (1979) have shown that injection of Ang II (100 μg/kg i v) into spinalized pigeons has no effects on shivering This experiment performed to alleviate the putative effects of low BP on muscle blood flow in spinal pigeons shows that the suppression of shivering by Ang II in intact pigeons is mediated by a mechanism involving CNS structures and not by direct effects on muscle blood flow

The exact mechanism by which baroreceptor activity affects shivering is not clear The pathways by which the rise and fall of BP mediate their effects are not necessarily the same Besides the systemic effects of baroreceptor stimulation a wide range of physiological functions including e g gamma motor activity (Schulte et al 1959) is influenced by the baroreflex The rapid onset of the responses suggests that a direct inhibition of efferent motor pathways controlling shivering is involved instead of systemic influences Mott (1963) too concludes that the baroreflexive effects are mediated by inhibition of motoneurons It is however known that a reduction of BP and the resultant decrease in baroreceptor activity induces an extremely pronounced vasoconstriction in skeletal muscle resistance vessels (see Kirchheim 1976) The ischemic effect of the vasoconstriction might have some influence on shivering in these experimental conditions

Baroreflexes are often considered to be inert on skin blood flow (see Kirchheim 1976) However the cold induced vasocon triction in the feet of the pigeon was abolished with rising BP (Fig 1) suggesting that baroreflexes affect skin blood flow in these conditions

The experiment with hypotensive pigeons (Fig 5) show that shivering can be enhanced by moderate baroreceptor stimulation This is in line with the results of Ishii & I hii (1960*a*) and Mott (1963) who observed that shivering could be enhanced in the rabbit by moderate stimulation of the baroreceptor nerves or by NA and adrenaline injections after eliminating part of the afferent baroreceptor input by cutting the respective nerves Furthermore Ishii & Ishii (1960*b*) noted that the deviation from normal body temperature needed to elicit shivering was greater in rabbits that were hypotensive These findings suggest that the normal input to CNS from baroreceptors has a perm- or facilitatory effect of shivering.

Another point pertinent to the present st[illegible] the integration of thermoregulatory and card[illegible] control mechanisms Because both systems [illegible] partly the same effectors a physiological [illegible] making is required when thermal and card[illegible] challenges are opposite Morishima & Gale [illegible] have demonstrated that thermal stimulat[illegible] hypothalamus in the baboon can result in [illegible] cardiovascular adjustments that are not affec[illegible] baroreflexes This can be interpreted as [illegible] porary suppression of baroreflexes by the [illegible] moregulatory need of the organism (see also [illegible] 1978 Coote et al 1979) The present results [illegible] cited evidence from mammals show that [illegible] rocal suppression of thermoregulatory re[illegible] may also occur in conditions where chan[illegible] baroreceptor activity are pronounced The [illegible] between these mechanisms is thus not strai[illegible] ward and it may depend on the magnitude [illegible] specific challenges presented In any case [illegible] ciprocal interactions suggest that thermal and [illegible] diovascular responses are controlled in an [illegible] grated fashion This conclusion is substanti[illegible] the fact that the same supramedullary sites [illegible] involved in temperature regulation also partic[illegible] in the integration of baroreflexes at least in [illegible] mals (e g Hilton & Spyer 1971)

We conclude that both a rise and a fall in [illegible] inhibit shivering thermogenesis in the pigeon [illegible] that NA inhibits shivering through a barorefle[illegible] loop and also through another possibly direct [illegible] tral route These effects probably expla[illegible] hypothermic effects of NA found in bir[illegible] thermore normal BP has a permi sive effect [illegible] shivering These interactions of thermoregula[illegible] and circulatory control mechanisms should be [illegible] sidered in thermoregulatory experiments and [illegible] procedures that affect the cardiovascular sys[illegible]

This research was partly supported by Oulun [illegible] Tukisäätiö We thank Hannu Rintamäki for helpful cus ions

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onal blood flow during reactive hyperemia nine myocardium as determined by local nout of Xenon 133

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HAUNSØ S & AMTORP O Regional blood flow during reactive hyperemia in canine myocardium as determined by local washout of Xenon-133 Acta Physiol Scand 1980 110 283–293 Received 7 Feb. 1980 Department of Cardiology Copenhagen County Hospital, Gentofte, Denmark

The mechanisms regulating vascular tone in the myocardium were studied in open-chest anesthetized dogs by occlusions of the left anterior descending coronary artery (LAD) for 3 to 600 s. Cumulative xcess blood flow (flow in excess of control flow) and repayment of flow debt (cumulative xcess blood flow divided by blood flow deficit) were calculated sing local injections of Xenon-133 for blood flow measurements Release of vascular occlusion following 3 s of ischemia was not associated with any measurable hyperemia Cumulative excess blood flow increased with increasing duration of ischemia from 5 to 600 but the increment in xcess flow per unit extension of the occlusion time showed considerable decline. Blood flow in excess exceeded blood flow debt incurred during the occlusion of 10 duration of 161% with prolongation of ischemia to 600 repayment of flow debt declined markedly to about 10% Oxygen lack in the tissue elicited by perfusion of LAD—for 10 with constant perfusion rate—with deoxygenated blood produced a fall in peripheral coronary resistance of about 40% which closely corresponds to the fall in resistance observed after period of LAD occlusion of similar duration The results lead to the conclusion that vasodilator metabolites formed in the tissue during periods of arterial occlusion are of prime importance for the fall in the tone of the vascular smooth muscle cell occurring in the post-occlusion period. The findings argue against myogenic component in this response

Key ords Xenon washout, cumulative excess blood flow autoregulation peripheral coronary pressure, deoxygenated blood vascular resistance

the circulation to tissue is released after a l of arrest blood flow is considerably in- ed and then returns to resting value after some es. This phenomenon of reactive hyperemia een used in several experimental approaches study factors determining vascular tone Blatt 1926, Montgomery et al. 1934 Abraham- t al. 1941 Green & Wegria 1942, Folkow 1949 rson 1956 Shepherd 1964 Lindbjerg 1966 ien & Sejrsen 1972, Henriksen et al. 1976) studies have gained from quantification of the ic hyperemia by measurement of cumulative s blood flow during hyperemia and repayment low debt incurred during periods of vascular usion

revious studies from myocardial tissue ha e n based upon measurement of absolute blood flow recorded from a major coronary artery by the aid of a rotameter or an electromagnetic flowmeter respectively The results so far obtained suggest that the response of the vascular bed in the myocardium to acute arterial occlusion is due partly to myogenic partly to metabolic mechanisms

The purpose of the present study was to reinvestigate the characteristics of reactive hyperemia in the intact canine myocardium using a local Xenon-133 washout technique (Lassen 1967 Haunsø et al 1979) which measures regional blood flow per gram of tissue The effect of occlusion of left descending coronary artery (LAD) lasting from 3 to 600 s upon the reactive hyperemic response was studied In other experiments the relaxation of the coronary resistance vessels in response to pure hypoxia was examined by measurement of peripheral coronary

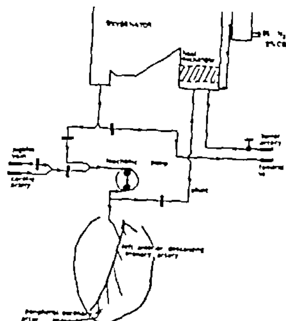


Fig 1 The experimental preparation used to study the influence of oxygen lack upon the coronary vascular bed (for further explanation see text)

artery pressure during and following perfusion of LAD in 10 s with deoxygenated blood at a constant perfusion rate The results indicate that vasodilator metabolites formed during myocardial ischemia are responsible for the post-ischemic hyperemia while myogenic mechanisms play little or no role

METHODS

General experimental procedur

Studies were performed on 18 dogs weighing between 19 and 30 kg. Anesthesia was introduced by i v injection of thiomebumal-sodium (Leopental®) 1 5 mg per kg and respiration was maintained with oxygen and nitrous oxide through an endotracheal tube u ing a respirator I v injections of fentanyl (Haldid®) 0 1 mg and pancuronibromidum (Pavulon®) 2 mg were given when required

A pigtail catheter was introduced through a femoral artery and the tip was placed in the ascending aorta. A Swan-Ganz flow directed thermodilution catheter (model 600855-22-6 F) was inserted through the right external jugular vein with the tip placed in the pulmonary artery Cardiac output was measured by injection of 5 ml 5% dextrose solution at 0°C into vena cava superior and calculated on a Devices (R 3750) cardiac output computer Through the left common carotid artery a catheter tip pressure transducer (Statham P-866) was placed in the left ventricle The pressure signal was amplified and differentiated in a modified Elfab (K4 BMC) pressure monitor The ventricular pressure P its first derivative dP/dt and the aortic pressure wave form were recorded on an Elema Mingograph 34 at a paper speed of 100 mm/s. Peak dP/dt was measured at the time just before opening of the aortic valves Aortic and pulmonary blood pressure re-corded continuously by means of a Statham tr[illegible] (model P 23 Db) and together with ECG recorded [illegible] U V recorder (model 5656) ABEM Body tem[illegible] was maintained by external heating and controlled [illegible] thermo couple thermometer placed in the rectum [illegible] oxygen saturation oxygen and carbon dioxide [illegible] pH and hematocrit were recurrently measured [illegible] thoracotomy was made and the pericardium was [illegible] and sutured to the thoracic wall in order to [illegible] changes in the position of the heart due to [illegible] movements Atelectasis was minimized by 3 cm [illegible] tinuous expiratory positive pressure

Heart preparations. (*a*) the left anterior des[illegible] coronary artery (LAD) was isolated about 3 cm fr[illegible] origin and silk snare or a pneumatic occluder wa[illegible] around the artery to be used for occlusions A small anterior branch of LAD was cannulated [illegible] a poly[illegible] chloride heparin filled catheter (o d. [illegible] mm) and peri[illegible] coronary artery pressure was measured by means [illegible] Millar PC 350 tip catheter (o d 1 5 mm) with the tip inside the polyvinyl catheter The ischemic [illegible] area following coronary occlusion was about 1[illegible] [illegible] entricular mass (*b*) LAD was isolated about one c[illegible] its origin After the dog had been anticoagulated heparin (400 U/kg) LAD was cannulated for per[illegible] with either arterial blood or blood with low oxygen [illegible] centration (cf Fig. 1) Perfusion at a constant [illegible] achieved by a peristaltic rotary pump During [illegible] conditions this was supported with blood from the [illegible] carotid artery (pO_2 about 120 mmHg) By means of [illegible] the blood supply to the pump could be [illegible] deoxygenated blood from an oxygenator (Polystan [illegible] term VT 2000) aerated with a gas mixture of 95 [illegible] 5% CO_2 The oxygenator was fed [illegible] blood from [illegible] femoral artery During LAD perfusion [illegible] carotid [illegible] oxygenator blood was returned to a femoral vein [illegible] switching to LAD perfusion with blood from the oxygenator blood from the carotid artery was [illegible] into a jugular vein. The coronary perfusion pressure [illegible] proximately equalled systemic arterial blood pressure (within 10 mmHg) Peripheral coronary pressure [illegible] measured as described abo e

Measurement of local blood flow

Myocardial blood flow in the region subjected to [illegible] was determined before during and following [illegible] occlusion using the local Xenon-133 washout technique Five μl of Xenon-133 (10 mCi/ml or 40 mCi/ml) [illegible] in sterile saline (obtained from the Radiochemical Centre Amersham England and AB Atomenergi, Studsvik Sweden) was injected into that part of the myocardium [illegible] the left ventricle which could be subjected to ischemia The depth of the local injection (5 mm) was secured [illegible] of a collar on the needle

The gamma radiation of Xenon 133 was monitored [illegible] scintillation detector with a thallium activated so-[illegible] iodide crystal placed 5 cm inside the opening of a [illegible] lead collimator The crystal was positioned at a distance of 14 cm above the anterior wall of the exposed left ventricle and collimated to obtained count rates from an area around the injection site [illegible] an amplifier [illegible]

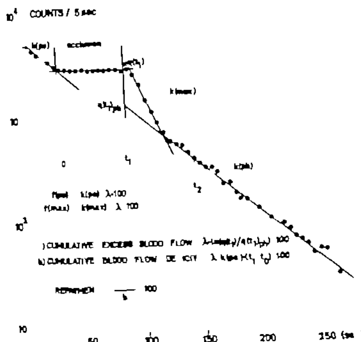


Fig 2 Representative washout curve following 60 of vascular occlusion recorded after intramyocardial injection (depth 5 mm) of 5 μl of Xenon-133 From the clearance curves washout rate constants before during and after reactive hyperemia were obtained and excess cumulative blood flow and repayment was calculated as illustrated

…mark, Denmark, RH 17 237) only gamma emission the 81 keV peak of Xenon-133 was recorded The … connected to digital ratemeter and the counts accumulated over periods of 1 or 5 The counting activity began immediately after each local injec… indicator

…ing experiments ere performed

… 10 dogs LAD was occluded for periods of 10 to … with at least 15 min interval between individual … ments In four of the dogs measurements were … using intramyocardial injection of 5 μl of … 133 (50 mCi/ml) in order to achieve high count … respect to background activity during the washout … hyperemia periods Results were similar to those … after intramyocardial injection of 5 μl of Xenon- … mCi/ml)

… In one dog measurements as described above were … after periods of vascular occlusions performed in … sequence (600 to 10)

… In three dogs peripheral coronary pressure and blood … measured following occlusions of LAD lasting … (means ± S D = 9), 5 3±0.2 (means ± … 11), and 7 7±0 3 (means±S D = 11), respec…

… In two dogs LAD was pump perfused with arterial … constant flow rate ensuring that peripheral … pressure equalled systemic arterial pressure … perfusion of LAD was then abruptly switched to … perfusion—for 10 with unchanged flow rate—with deoxygenated blood from the tracorporal circuit Peripheral coronary artery pressure was continuously re corded

( ) T assess the possible influence of blood clotting or platelet thrombosis upon the response to vascular occlusion, two additional dogs were heparinized with heparin sodium (400 U/kg) administered intravenously

Aortic and pulmonary artery pressures, heart rat car diac output and peak dP/dt were measured before during and following periods of ischemia. Experiments were discarded if these parameters during the occlusion or during the post-occlusion period differed more than 10% from the pre-occlusive values Changes in peak dP/dt of such magnitude were not observed in any of the occlusion xperiments.

Calculations

Count rates of Xenon-133 corrected for background activity were plotted in semilogarithmic diagrams. A typical washout curve from 60 occlusion xperiment is shown in Fig. 2 Assuming homogeneous perfusion, no arterial recirculation or veno-arterial shunting by diffusion and flow limited washout of the indicator the following parameters were calculated from the curves.

1 Pre-occlusive f(po) and post-hyperemic blood flow f(ph) were calculated in accordance with th equation

$f\ k\ \lambda\ 100\ (ml\ min^{-1}\ (100\ g)^{-1})$

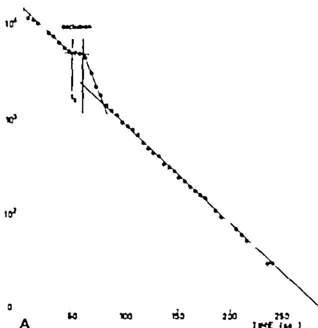


Fig 3 Examples of Xenon-133 washout curves [?] 10 s (A) and 1?0 s (B) of vascular occlusion The parts of the curves have the same shape with [?] slopes. About 5 s after vascular occlusion the [?] curves are almost horizontal After release, the [?] chemic disappearance rate of Xenon-133 [?] increase with a steep initial slope of the curves [?] acceleration period of 5 to 10 s duration depending duration of ischemia. The disappearance rate of [?] 133 returned to pre-occlusive values as determined the last part of the washout curves [?] monoexponential course

where k is the rate constant mea ured from the washout curve and λ is the tissue to blood partition coefficient in ml (g) (Kety 1951) The λ values in all experiments were obtained as slopes of the regression lines calculated according to the least square method using logarithmical ly transformed count rates corrected for background ac tivity The λ values used were those given by Tonnesen & Sejrsen (1967) for skeletal muscle corrected from mea ured hematocrit values

Blood flow values during reactive hyperemia were defined as follows

Maximum blood flow f(max) wa calculated from the λ value corresponding to the in tial steepest part of the Xenon 133 washout curve following release of arterial occlusion In the experiment portrayed in Fig blood flow was stopped at $t = t_o$. The duration of the hyperemic period (t –t) was taken as the period from release of vascular occlusion t = t until blood flow had decreased to pre-occlusive le el at time t

Cumulative excess blood flow and repayment of flow debt per 100 g of tissue was calculated as described by Kristensen & Henriksen (1977) cf equations in Fig

3 Cumulative excess blood flow $V_B$excess which equals integrated blood flow during reactive hyperemia minus integrated pre-occlusi e blood flow corresponding to the duration of the reactive hyperem was calculated in accordance with the equation

$$V_B\text{excess} = \lambda \ \ln (q(t_{...})) \ 100$$

where $q(t_i)$ is the activity at the time of release of ascular occlusion and $q(t)_{...}$ is obtained by retropolating the activity versus time curve from t k t_e (This calcul gives cumulati e excess blood flow whether the [?] flow is constant or varies in the period t to t)

4 Repayment (R) of flow debt which equals cum[?] excess blood flow divided by pre-occlusive blood [?] times duration of ischemia was calculated in per [?] accordance with the equation

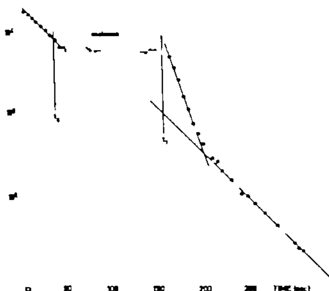
$$R = \frac{V_B\text{excess}}{f(po)\ (t_r - t_o)} \ 100$$

In the analysis of the results Student t-test was [?] to determine the significance of differences [?] paired observations in each group and unpaired [?] vations between different groups of experiments [?] of significance was chosen 0.05

RESULTS

In Fig 3 A the artery was occluded for 10 s [?] occlusive blood flow was 97 ml min [?](100 g)[?] [?] release of the occlusion there was a short dela[y] thereafter a rapid increase in coronary blood [?] Thereafter blood flow decreased to the [pre-] occlusive value within about ?0 s

In Fig 3 B the artery was occluded for 1[?] Pre-occlusive blood flow was 104 ml min [?] g) It appears that the washout of Xenon-133 delayed about 10 s a release of the vas[cular]



on. Thereafter the post-ischemic washout of 133 showed a rapid increase resulting in a ...low n-slope The disappearance rate of the ...or returned to the pre-occlusive value within ...determined from the last part of the washout ...hich follows a monoexponential course In ...eriments the activity ...erans time curves be ...orizontal following vascular occlusion after a ...deceleration period (5–10 s) ... the initial of the occlusion The washout rate after re... of the vascular occlusion showed ... rapid in... ...hich occurred after an acceleration period ...0 s duration depending upon duration of is... ...a

...results obtained in 69 expts performed on 13 ...group ... +c) are summarized in Table 1 The ...est rate constant obtained before vascular ...tion, k(po) and rate constants k(ph) obtained ...ds the end of the post-hyperemic period did ...ffer significantly ($P>0.05$). Maximum blood ...increased significantly when duration of is... ...ia was prolonged from 5 to 30 s ($P<0.05$) ...eration of duration of ischemia t 60 120 240 ...600 ... did not cause ... further increase in ...imum blood flow The calculated cumulative ...ia blood flows increased continuously with ...tion of vascular occlusion from 5 to 600 s.

Repayment f flow debt following 10 s of ischemia amounted to 260% Following 30 s of ischemia repayment was reduced to around 100% and further marked reduction occurred with longer ischemic periods viz. to less than 10% following 600 s of ischemia.

No difference could be observed between the results obtained in 10 dogs (group a) and those btained in one dog (group b) where the vascular occlusions of different duration were performed in the opposite sequence. Repayment during reactive hyperemia amounted in the latter to about 8% 47% 7?% 130% and 299% following 600 120 60 30 and 10 s of ischemia.

I the xperiments with brief occlusion periods (less than 10 s) and measurements of peripheral coronary pressure (group c) reactive hyperemia was not measurable following 3 s of occlusion. I all these expts the coronary arterial pressure distally to the occlusion fell within 1 to 2 beats by 60 to 80 mmHg to a level of 10 to 20 mmHg, and rose rapidly to pre-occlusive values when the occlusion was re leased (Fig. 4)

In 10 expts performed on dogs (group d) where LAD was perfused for 10 s with deoxygenated blood (pO_2 = 16 to 22 mmHg) peripheral coronary artery pressure fell from 103 4±9.2 mmHg (means

Table 1 *Reactive hyperemia characteristics in 13 dogs obtained from increasing lengths of* [illegible] *occlusion times*

In columns are shown (1) the length of occlusion time (2) the number of measurements corresponding to each [illegible] period (3) the blood flow values measured from the washout curves of Xenon-133 before vascular occlusion (4) [illegible] flow values measured from the initial steep slope of the Xenon-133 washout curve during reactive hyperemia (5) [illegible] duration of reactive hyperemia (6) blood flow values measured from the washout curves during the late post-hyper[illegible] period (7) the values of excess cumulative blood flow in the hyperemic period and (8) the values of repayment calculated from excess blood flow in per cent of blood flow debt Figures are mean values ±1 S.E.

Ischemic period (s)	Number of measurements (n)	Pre-occlusive blood flow f(po) ml (100 g) min	Maximum blood flow f(max) ml (100 g) min	Duration of reactive hyperemia (s)	Post-hyperemic blood flow f(ph) ml (100 g) min	Excess cumulative blood flow V_{excess} ml (100 g)	Repayment (%)
5	11	110 3± 7 9	169 8±12 1	33._±1.8	108.5±7.5	14 5± 1.3	[illegible]
8	10	111.3± 8 9	187 6±15 1	36.5±1 9	110 3±8 7	26 0± 1 9	[illegible]
10	10	114.8± 7 8	221 7±11.8	39 1± 0	10_5±7 0	39.8± 5 7	[illegible]
30	10	109 8± 4.3	_52.6±13 1	43.8±3.3	106 7±3.3	60.8± 5.5	[illegible]
60	8	99.8± 6 1	71.8±13 7	53.8±4 3	90.5±8.0	71.5± 7 1	[illegible]
120	7	10_0± 5 4	_56.6±14 3	65 1±1 7	10_2±9.3	71.5± 9 5	[illegible]
240	7	109.5± 4 9	44 7±11._	68.0±4 4	105.5±4._	81.9±11.5	[illegible]
600	6	1_3 4±10 8	264 7±19 1	69 6±3.8	119 6±9.5	89.8± 8 1	[illegible]

± S D) to 57 6±9 9 mmHg (means ± S D) with a short delay of 3 8±0.5 s (means ± S D) No changes in the rate of washout of Xenon-133 occurred in agreement with the fact that flow rate generated by the rotary pump was unchanged on switching from oxygenated to deoxygenated blood and back. Regional blood flow was calculated to 106±38 ml min (100 g) (means ± S D) The fall in peripheral coronary vascular resistance amounted to 41%

The results obtained with two heparinized dogs (group e) did not differ from those of unheparinized indicating that blood clotting and/or platelet thrombosis were not substantial factors in the hyperemic response

DISCUSSION

Estimation of blood flow in control state and during reactive hyperemia from the washout of a freely diffusible indicator was used to study the mechanisms regulating vascular tone in the myocardium The local washout technique of Xenon-133 used for calculation of regional blood flow and its application to myocardial tissue has been discussed in detail in a recent study (Haunsø et al 1979)

A crucial point in the calculation of blood flow is whether the tissue to blood partition coefficient of Xenon-133 (λ_{Xe}) changes during experiments due to tissue damage The conclusion that λ_{Xe} does not change during the experiment is based upon the observation that no difference could be de[illegible] between the calculated maximum post-[illegible] blood flow values obtained when periods [illegible] chemia were changed from 30 to 600 s or [illegible] Furthermore blood flow values calculated [illegible] occlusive periods and late post-occlusive [illegible] did not differ significantly indicating that [illegible] dial tissue was not altered in composition [illegible] appreciable extent

The myocardial reactive hyperemic [illegible] noted in the present study was similar to that [illegible] tained by use of electromagnetic flowmeter [illegible] local washout of Xenon-133 (residue [illegible] shows initially after release of the vascular [illegible] clusion a short delay of 5 to 10 s duration [illegible] vascular bed in the counting field is filled [illegible] blood thereafter Xenon-133 activity vs time [illegible] shows a fall representative of outflow of blood [illegible]

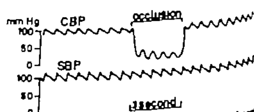


Fig 4 Effects of 3 s occlusion on peripheral cor[illegible] blood pressure (CBP) and systemic arterial blood pr[illegible] (SBP) Note that CBP is reduced by 60 to 80 mmHg [illegible] 1 to beats following the occlusion and rises rap[illegible] pre-ischemic blood pressure level when the occlu[illegible] released

...ion. The electromagnetic flowmeter (inflow ...) measures initially after release of vascular ... the inflow of blood into a partly empty ... bed, and then registrates blood flow ... the tissue mass corresponding to the supply artery. The observed delay in the washout of ...133 corresponds to the electromagnetically ... delay (5 to 10 s) of attaining peak reactive ... flow rate.

...augmented blood flow of reactive hyperemia ... myocardium following periods of vascular ... is due to relaxation of vascular smooth ... cells in coronary resistance vessels, i.e. ar... and small arteries. The mechanisms under... the relaxation have been discussed earlier ... and metabolic mechanisms have been ...d, but consensus has not been reached ... & Gregg 1960, Coffman & Gregg 1961, ... et al. 1964, Olsson & Gregg 1965, Gellai et ... Eikens & Wilcken 1973, 1974, Bache et al 1974, Olsson 1975, Giles & Wilcken 1977) ... to the myogenic hypothesis, vascular ... muscle cells will change their tone in re... to changes in transmural pressure (Bayliss, Folkow 1949, Patterson 1956, Sparks & Bohr, Folkow 1964). This response is claimed to be ... than a few seconds and to be completed ... min. The metabolic hypothesis implies ad... of blood flow to the metabolic demand of ...ocardium, but the exact mechanism coupling ... flow and tissue metabolism is unsettled. It is ... known that ... number of vasodilator ...olites accumulate in the myocardium during ..., adenosine, bradykinin, carbon dioxide, ...olamines, histamine, lactic acid, potassium, ...glandins and serotonin (Berne 1964, Olsson, Olsson 1975, Wayne et al. 1975, Rubio & ... 1975, Murray et al. 1979).

... the present experiments we have measured ...teral coronary artery pressure, estimated re... myocardial blood flow and calculated ...tive excess blood flow and repayment of ... debt incurred during periods of vascular ...sion. The occlusion reduced peripheral coro... artery pressure within 1 to 2 beats by 60 to ...mHg and the pressure returned immediately ...re-occlusive level after release of vascular ...sion (Fig. 4). Maximum blood flow during ... hyperemia increased with increasing length ... vascular occlusion up to 30 s. Further prolon... of the duration of ischemia did not increase maximum blood flow any further indicating that the resistance vessels in the coronary vascular bed were maximally dilated following 30 s of ischemia. This result is consistent with those reported by Coffman & Gregg (1960) and by Olsson (1975), but at variance with those obtained by other investigators (Olsson & Gregg 1965, Reneman & Spencer 1972, Bagger 1977, Dunn et al. 1979) who noted maximally vasodilatation to occur following 10 s of occlusion. It should be added that the present study did not include occlusions of between 10 and 30 s duration.

The cumulative excess blood flow in the myocardium following periods of occlusion of the supplying artery increased with increasing duration of the occlusion, but the increment in excess blood flow per unit extension of the occlusion time declined considerably. These events may well be accounted for by accumulation of vasodilator metabolites in the tissue approaching an upper concentration with increasing duration of the occlusion time. During hyperemia these metabolites will be eliminated by washout, cellular uptake or/and metabolic conversion at a concentration dependent rate, e.g. first order kinetics.

Following brief occlusions of the coronary artery maximum blood flow and excess blood flow were considerably reduced and when occlusion time was shortened to 3 s, no increase in post-occlusion blood flow was detectable. The rate of change in perfusion pressure head was similar to that used by Sparks (1964) in human umbilical artery strips and might uncover a myogenic response of smooth muscle cells in coronary resistance vessels if existing. The results argue against a myogenic response playing an important role in post-occlusion hyperemia. The effect of metabolic factors on vascular tone was further studied by examining the influence of oxygen lack upon relaxation of coronary resistance vessels. Experiments were performed in which ... coronary artery was perfused for 10 s with deoxygenated blood at ... constant flow rate. With an abrupt fall in arterial oxygen tension from about 120 to about 20 mmHg no changes in the rate of washout of Xenon-133 occurred, but a fall in peripheral coronary pressure to about 41 % was recorded within 4 s indicating a similar fall in vascular resistance. It is noteworthy that this decrement in vascular resistance closely corresponds to the observed decrement in vascular resistance following 10 ... of vascular occlusion. I... the short interval of

4 s from tissue perfusion with deoxygenated blood until a fall in peripheral coronary pressure was recorded metabolic disturbance in the myocardium was not sufficient to produce measurable vasodilatation Correspondingly 3 s of vascular occlusion was apparently too short a period to induce oxygen lack resulting in significant release of vasodilator metabolites —while it should be sufficient to induce a myogenic response

The high value for repayment following brief occlusions may be elicited by a fast rate of accumulation of vasodilator metabolites shortly after an occlusion an accumulation that may be enhanced in the post-occlusion period by the transient increase in contractility force in the myocardium induced by ischemia of short duration (Theroux et al 1974) It is possible that vasodilator metabolites are formed at a reduced rate as myocardial contractions in the ischemic area cease when the arterial occlusion exceeds a certain duration If it is so it may at least contribute to the marked fall in repayment observed with increasing duration of ischemia above some 60 s

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e and chewing as stimuli for the secretion of amylase
the parotid gland of the rabbit

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GJÖRSTRUP P. Taste and chewing as stimuli for the secretion of amylase from the parotid gland of the rabbit. Acta Physiol Scand 1980, 110: 295–301. Received 13 Febr. 1980. ISSN 0001-6772. Institute of Physiology and Biophysics, University of Lund, S-223 62 Sweden

Reflex secretion of amylase was studied in response to feeding pellets and carrots. Section of the ipsilateral glossopharyngeal nerve but not of the lingual nerve reduced the secretion of amylase with carrots but not with pellets. The fluid secretion was unaffected by the nerve sections. Larger volumes were secreted with pellets than with carrots, indicating that the hardness of the food, via chewing, is the main stimulus for fluid secretion. Sweet, salty, sour and bitter taste stimuli did not produce any fluid secretion in concentrations normally used for gustatory stimulation in rabbits. In a background flow of parasympathetically secreted saliva sweet stimuli regularly caused a large increase in the amylase output, salty stimuli usually had a small effect and sour and bitter stimuli seemed to have only exceptionally a small effect. Citric acid in a high concentration caused fluid secretion, at the most at a rate of about 50% of maximum; this saliva had a high content of amylase. The results support the view that during feeding the sympathetic secretory nerves can be activated via both taste stimulation and chewing, while the parasympathetic activity is mainly influenced by chewing.

Key words: Rabbit, parotid gland, amylase secretion, reflex secretion, taste, chewing

ommon knowledge that gustatory stimulation kes secretion of saliva and the phenomenon ten extensively studied in both humans and ls, particularly in the parotid but to some x lso in the submaxillary gland (e.g. Lashley Chauncey & Shannon 1960, Kerr 1961, oula & Kawamura 1967, Suhara, Takashita & u 1967, Kawamura & Yamamoto 1978). With xceptions (Baxter 1930, 1931, Newbrun 1962) investigations have dealt with the effects on alivary flow rate only, and far less is known t the influence on the secretion of organic tonents, the information available suggests that not necessarily linked to the rate of fluid secre Similarly, the flow rate has usually been the are used to evaluate the effects of chewing on alivary secretion (Lashley 1916, Kerr 1961). t is known that human parotid saliva obtained chewing contains amylase (Newbrun 1962) ce the kind of stimuli that lead to parasympathetic activation, which is the main cause of the l secretion (see Emmelin 1967), are better wn, than how activation of oral receptors may e increase the sympathetic activity: these nerves seem to have the principal control over the secretion of proteins, especially investigated as the secretion of amylase in rats (Schneyer 1974, see Schramm & Selinger 1975, Garrett & Thulin 1975).

Experiments with electrical stimulation of the secretory nerves to the parotid gland of the rabbit indicate that in this gland a separate control of amylase and fluid secretion could be possible (Gjörstrup 1979, 1980). In conscious rabbits provided with parotid fistulae (Gjörstrup 1980) the feeding of carrots brought about a saliva with an amylase concentration 3–4 times as high as with pellets. In spite of this difference the two kinds of food stimulated to about the same high output of amylase, due to the much higher flowrates with pellets than with carrots. Thus the results indicated not only the existence of a separate activation of the sympathetic and parasympathetic nerves, but also the possibility that more than one afferent nervous pathway may be involved in the control of amylase secretion, which has now been further investigated.

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METHODS

Surgical procedures

17 rabbits weighing –2 8 kg were used All surgery was done under pentobarbitone anesthesia, 40 mg/kg given through a lateral ear vein, sometimes ether was added To provide a salivary fistula the right parotid duct was exposed by an incision below the eye and cannulated with a polyethene tubing (I D 0.58 mm × O D 0 965 mm) that was bent subcutaneously backwards over the masseter muscle and led out through an incision behind the right ear (Gjorstrup 1979*b*) The patency of the system was tested by injections of methacholine 3–5 μg/kg i v On the same occasion 10 of the animals had a second tubing of the same bore implanted with one tip entering into the mouth of the rabbit By using the incision made for the cannulation of the salivary duct a wide bore injection needle was pushed from behind between the mandibular ramus and the masseter muscle in an anterio-medial direction Simultaneous intraoral palpation over the buccal region and the palate made it possible to guide the needle to penetrate the oral mucosa behind the last upper molar on the right side The tubing was slipped through the needle leaving –3 mm in the mouth and pointing forwards. The needle was removed and the tubing then led out behind the right ear beside the salivary cannula. 3–5 days later either the ipsilateral chordo-lingual nerve in 4 rabbits or the glossopharyngeal nerve in seven rabbits was cut. The chordo-lingual nerve was cut 5 mm laterally to the submaxillary duct and the glossopharyngeal nerve was sectioned near the exit from the scull where it branches In two of the animals the section of the glossopharyngeal nerve was done a few days after the chordo-lingual nerve had been cut in the other cases the two kinds of nerve sections were done in different animals.

Feeding experiments

The animals were fed either pellets or peeled carrots at intervals of 24 hours, on each occasion it was tried to feed the animal both kinds of food and from time to time to reverse the order in which it was given During feeding the external end of the tubing coming from the salivary duct was arranged for saliva to drop into small test tubes, which were changed every or 3 min. Each day 5–8 samples of saliva could usually be collected The animals were calm during testing and did not show any signs of discomfort or impaired chewing due to the implanted tubings. A detailed description of this kind of experiment has been given elsewhere (Gjörstrup 1980)

Taste experiments

Through the polythene tubing implanted into the mouth various taste stimuli could be introduced without having to strain the animals which most often is the case when substances are to be given orally The following substances (in reagent grade and dissolved in distilled water) were used as taste stimuli Sucrose (0.5 M) sodium chloride (0.5 M) hydrochloric acid (0 02 M) quinine chloride (0 02 M) citric acid (0.5 M) and freshly pressed carrot juice The taste solutions were injected at a speed of 5 ml/min which seemed sufficient for a good and continuous filling of the mouth since the animals usually drooled slightly in spite of licking and swallowing [illegible] solutions were generally given into the mouth [illegible] with the exception of citric acid and carrot juice [illegible] were only given for 3 and 4 min at a time, repeated During the infusion of the taste solutions saliva was [illegible] ly collected in 1 or min periods, [illegible] samples collected over 3 min. One min of [illegible] always allowed before sampling of saliva [illegible] taste solutions were given either when the parotid was quiescent or when it was secreting at a rate of [illegible] μl/min This fluid secretion was brought about by [illegible] grade injections of 0.3–0.4 ml of eserine solution [illegible] into the gland through the tubing in the salivary d[illegible] Emmelin et al 1954). The secretion in response [illegible] was allowed to build up for about 1 min then [illegible] samples of saliva were collected during [illegible] solutions were introduced The periods [illegible] tions were finished with rapid rinsing by [illegible] mouth with 5–10 ml of distilled water and then [illegible] min elapsed before control samples of saliva [illegible] collected

Amylase and flow rate measurement

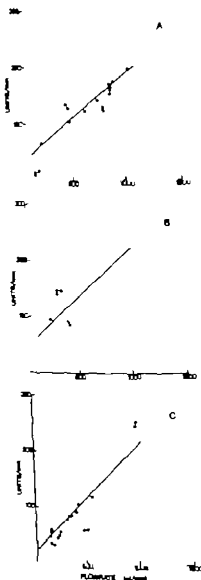
The samples of saliva were stored in deep freeze [illegible] assayed according to the method described by [illegible] (196[illegible]) In this method 1 unit of amylase is equal [illegible] the amount that liberates reducing groups corresp[illegible] to 1 μmole maltose formed each minute at 25°C [illegible] of saliva of 50 and 100 μl were used and diluted [illegible] times before processing The amylase secretion [illegible] pressed as the concentration of amylase in units/[illegible] the output of amylase in units/min The obtained [illegible] of saliva were measured with micropipettes, which [illegible] lowed measurement down to 10 μl with an accura[illegible] ± [illegible]

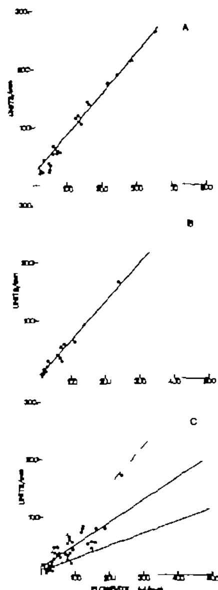
Statistics

Statistical evaluation was performed with linear regre[illegible] sion analysis and Student's *t*-test for unpaired [illegible] tions *P* values less than 0.05 were considered significant Mean values are expressed as mean ± S E

RESULTS

Figs 1*a* and 2*a* show as has earlier been sh[illegible] (Gjörstrup 1980) that the salivary flow [illegible] the parotid gland when pellets are fed is 3–4 [illegible] larger than that obtained with carrots but [illegible] spite of this difference the gland is stimulated [illegible] produce an amylase secretion which for both [illegible] of food can reach the same high magnitude [illegible] 250 units/min This amylase output remained [illegible] changed with both pellets and carrots after sec[illegible] of the ipsilateral lingual nerve carried out in 4 [illegible] bits (Figs 1*b* and 2*b*) Section of the ipsil[illegible] glossopharyngeal nerve in 7 rabbits diminished [illegible] output of amylase on feeding carrots, as indica[illegible] by the significant reduction in the regression [illegible] compared to that in the normal situation ($P<$[illegible]





! Amylase secretion in response to pellets is shown
a output versus the flowrate. The average secretion
ylase is show as the linear regressions. (A) Normal
vation (regressor Y=0 15 33 10 r=0 67·
001 45 (B) After section of the ipsilateral lingual
e (regressor Y 0 17x 49 17 r=0.71 P<0 001
7) (C) After section of the ipsilateral glossopharyngeal
e (regressor Y=0.19x 20 30· =0 72; P<0 001
7) Observations from 9 rabbits

Fig. 2. Amylase secretion in response to carrots as the output versus the flowrate the same rabbits as in Fig. 1 (A) Normal innervation (regressor Y=0 68x+14 79 =0 88 P<0.001 =49). (B) After section of the lingual nerve (regressor Y=0 65x+1 03 r=0.89 P<0 001 =19) (C) After section of the glossopharyngeal nerve (regressor Y=0 39x+12.70 =0 86; P<0.001 =54). In C the regressor under A (dotted line) and that obtained after preganglionic sympathectomy (broken line) from Gjörstrup (1980) have been inserted for comparison

. 2) while the output with pellets still could ch the magnitude obtained in rabbits with an act innervation (Fig. 1). This reduction in ylase output ith carrots after section of the glossopharyngeal nerve varied from animal to animal in some rabbits the reduction in amylase secretion was about 50% while it in other was less pronounced. After section of the lingual or the glos-

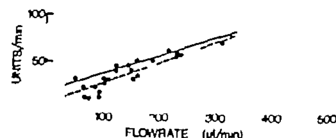


Fig 3 Secretion of amylase in response to retrograde injections of eserine 0.3–0.4 ml of a solution with a concentration of 1 mg/ml The output is shown versus the flowrate To compare with the level obtained after preganglionic sympathectomy (broken line) from Gjörstrup (1980) linear regression analyse was performed $Y=0.19x+19.79$ $r=0.50$ $P<0.01$ $n=40$

sopharyngeal nerve flowrates as high as those observed in intact animals were still obtained i e around 400 µl/min with carrots and around 1000 µl/min with pellets

The salivary fluid secretion in response to taste stimulation with sucrose sodium chloride quinine chloride hydrochloric acid and citric acid is summarized in Table 1 Apart from citric acid the power to provoke fluid secretion was poor characteristically the salivary flow response to citric acid increased gradually and did not attain a high rate until a distinct behaviour of aversion was seen The poor ability of the taste stimuli to cause fluid secretion made it necessary to provide a salivary flow when testing their effects on the amylase secretion By retrograde injections of eserine into the glands salivary flows within the range seen when feeding carrots were obtained (Fig 3) the corresponding amylase output was low being at the level recorded after preganglionic sympathectomy in a previous series of expts (Gjörstrup 1980) When in this situation sucrose was administrated into the mouth it caused a marked increase in the amylase content of the ongoing secretion while the flow rate remained unchanged On all occasions when sucrose was tested the amylase secretion was increased to at least the double compared to controls ($P<0.001$) and most often much higher (Fig 4*a*) At the continuous administration of sucrose into the mouth the amylase secretion regularly kept high for at least 2 min and then started to decline in some animals but could in others keep up for the whole period of 5 min The highest output of amylase in a single sample with sucrose was 343 units/min collected at a flowrate of 180 µl/min The amylase secretory effects in response to sodium chloride are summarized in Fig 4*b* With this stimulus amylase secretion was more variable in most cases a real increase was seen about 10–20% of controls in some sodium chloride failed to increase the amylase output and finally in two animals an increase to about the double was recorded When the mean of controls was compared to that of samples collected with sodium chloride the increase was found to be significant With sodium chloride the highest output of amylase was 159 units/min obtained at a flowrate of 215 µl/min Hydrochloric acid and quinine chloride administered to and 4 rabbits respectively did not cause any increase in the amylase output In controls the output varied between 11 and 4 units/min while it during test with hydrochloric acid and quinine chloride between 18 and 65 units/min with the exception of one sample where quinine chloride was found to cause a release of 99 units/min Carrot juice tested in 3 animals increased the amylase output by about 100% with the highest output at 154 units/min at 145 µl/min these results are shown in Fig 4c

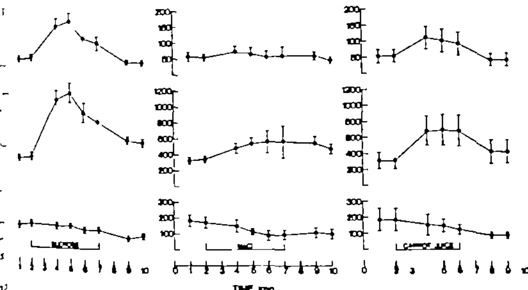
Citric acid the only taste stimulus to evoke fluid secretion also caused the release of amylase Amylase secretion was measured on 4 occasions with an average of 683±8 units/ml and a corresponding output of 47±30 units/min The highest output obtained was 317 units/min at 450 µl/min

DISCUSSION

The results clearly show that gustation is a powerful stimulus of amylase secretion in the rabbit From the experiments with feeding carrots after section of the lingual or glossopharyngeal nerves on the ipsilateral side of the cannulated gland it may be inferred that the information is conveyed in the glossopharyngeal nerve The unchanged level of

Table 1 *Volume secretory responses to various gustatory stimuli Injected intraorally continuously for 3–5 min at a rate of 5 ml/min*

Substance (molar concentration)		Flow rate (µl/min)	Number of experiments
Sucrose	(0 5)	<10	4
NaCl	(0.5)	~20	6
HCl	(0 0_)	~10	3
Quininechloride	(0 0)	<10	3
Citric acid	(0 5)	363±5	7
Carrot juice	(–)	<10	5



f Amylase secretion as output and as concentration in response to intraoral administration of solution of sucrose M), sodium chloride (0 5 M) and carrot juice The solutions were continuously injected at rate of 5 ml/min through inserted polythene tubing emptying into the mouth. Since the taste stimuli in themselves only evoked fluid secretion, they ere administered in the presence of a fluid secretion obtained by injections of eserine into and one min before zero-time Samples of saliva were taken at one or two min intervals. Control samples were cted before and after administration of taste stimuli Saliva was not collected during the first min of administration of solution and not during the first min afterwards, due to deadspace and the time for the effects to reach steady state cut off at the end of the stimulation. Sucrose (each point mean ± S E. of 6–9 observations, Sodium chloride of 6–8 observations); Carrot juice (mean of 3 observations)

lase secretion after section of the lingual nerve accordance with experiments on decerebrate showing but scant secretion from the parotid d on activation of the lingual nerve on the same (Miller 1913) Further it is known that section he lingual nerv makes the submaxillary but not parotid gland supersensitive to sialogogue drugs Emmelin 1961). The reduction in amylase secre after section of the ipsilateral glossopharyngeal ve varied in extent from animal to animal and) indicate an influence from the intact contralat l side that a unilateral input in the glossopharyngeal or lingual nerve can lead to activation parotid or submaxillary glands on both sides has n demonstrated in experiments on several cies (Miller 1913 Lashley 1916, Babkin 1928 Emmelin 1961).

S eet taste stimuli regularly caused a great increase in the amylase secretion while the effect of It was less powerful and occasionally even lack g. bitter and sour as hydrochloric acid seemed to without effect. A further example of the power of sucrose as stimulus for amylase secretion was that it could evoke an output of amylase about as high as with isoprenaline injections, or as seen in normal animals reflexly secreting during feeding (Asking & Gjorstrup 1980 Gjorstrup 1980) An investigation in man indicates sucrose to be only slightly more effective than the other taste qualities in producing amylase secretion (Newbrun 196..) however for all stimuli the reported values are rather low Carrot juice although subjectively less sweet than 0.5 M sucrose solution, caused a fairly high output of amylase as expected from the reflex secretion of amylase when feeding carrots (Gjörstrup 1979*b* 1980)

The receptors innervated by the glossopharyngeal nerve of the rabbit have been shown to respond to all 4 basic tastes, using solutions of the same kind as in the present expts (Appelberg 1958, Yamada 1967). The concentrations of the solutions used in the present investigation, e cept for citric acid and carrot juice have in whole nerve preparations of the chordo-lingual and the glossopharyngeal nerves of rabbits gi en about the same response measured as the intergrated nervous activity (Beidler et al.

1955 Pfaffmann 1955 Yamada 1967) Recordings from single nerve fibre preparations in the chorda lingual nerve show all units to respond to the 4 basic tastes but at thresholds that for the single stimulus vary from one unit to another (Pfaffmann 1955 Beidler 1967) Thus it may be suggested that the prominent effect of sucrose on the amylase secretion is due to a specific pattern of discharge in the glossopharyngeal nerve that selectively activates the sympathetic nerves responsible for amylase secretion

The high amylase output by pellets which do not have a sweet taste strongly suggests that other sensations or activities than gustatory can evoke secretion of amylase An obvious explanation would be that chewing is the additional stimulus responsible for the activity in the sympathetic nerves in the rat chewing has been suggested as the main stimulus leading to amylase secretion (Schneyer 1974) and in man chewing of dental wax caused secretion of amylase but less than obtained by gustatory stimulation (Newbrun 1967) In the present experiments the assumption is further supported by the unchanged level of amylase output with pellets after section of the glossopharyngeal nerve Chewing as additional stimulus for amylase secretion may also serve to explain why the output with carrots after glossopharyngectomy was not reduced to the level obtained after preganglionic sympathectomy

Apart form citric acid the taste stimuli did not cause any secretion of fluid not even an increase in the flow caused by eserine Hence it may be concluded that taste in this species is of less importance for the secretion of fluid than is the case in man or the dog (Lashley 1916 Baxter 1930 1931 Chauncey & Shannon 1960 Kerr 1961) The small fluid secretory effects are in agreement with experiments on both the submaxillary and the parotid glands of the rabbit (Kawamura & Yamamoto 1978 Suhara et al 1967) compared to the volumes obtainable during feeding their recorded volumes in response to taste stimuli were very low The greater fluid secretory effects with pellets than with carrots agree as earlier discussed (Gjörstrup 1980) with chewing as the main stimulus responsible for fluid secretion (Lashley 1916 Kerr 1961) Mechanical stimulation of the oral mucosa and particularyly of the tongue is also konwn to cause secretion of saliva (see Babkin 1928) and the reflex was first electrophysiologically described in recordings from afferent and efferent fibres in the chorda tympani of the rat (Hellekant & Kasahara 1973 a b) however in the rabbit the reflex seems to be of little importance during feeding since the flow of saliva was unchanged after section of the lingual or the glossopharyngeal nerves

Citric acid may as stimulus for salivary secretion occupy a place apart from other taste stimuli, when given in high concentrations in order to elicit maximal secretory rates In the rabbit a 0.5 M solution was followed by signs of aversion which in view of the poor responses to gustatory stimuli may be the main cause for the secretion In the dog citric acid is shown to cause salivary flow rates at maximum (Emmelin & Holmberg 1967), which is suggested to be the case also in man (Kerr 1961) In the parotid gland of the rabbit on the other hand citric acid could at the most produce a secretory rate at about half the maximum for the gland (Gjörstrup 1980) and in the submaxillary gland of the rabbit the secretion to noxious stimuli are reported to be even less (Kawamura & Yamamoto 1977 1978) The high amylase secretion indicates a reflex activation of the sympathetic nerves in response to citric acid which has previously been demonstrated by Ohlin (1966) for fluid secretion in the submaxillary gland of the rat

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gradation of TRF immunoreactivity in rat hypothalamus trahypothalamic brain and pancreas

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KOIVUSALO F Degradation of TRF immunoreactivity in rat hypothalamus, extrahypothalamic brain and pancreas. Acta Physiol Scand 1980, 110 303–306. Received 15 Feb 1980. ISSN 0001-6772. Institute of Physiology University of Oulu, Finland

The endogenous TRF as measured in radioimmunoassays of chromatographically purified extracts of excised hypothalami, extrahypothalamic brains and pancreas was shown to be stable in intact organs and homogenates. However synthetic TRF added to tissue homogenates was degraded Sonication of the homogenates liberated endogenous TRF for degradation. Chromatographically purified tissue extracts of hypothalamus, extrahypothalamic brain and pancreas were degraded similarly by rat serum These results suggest that the major portion of endogenous TRF occurs in protected form in tissues and hence the physiologically effective amount of TRF must be very low when compared to its total content in tissues.

Key words TRF pancreas degradation

have previously shown that several neural gastrointestinal tissues after methanol extraction, chloroform washing and cation exchange chromatography contain immunoreactive TRF (Leppäluoto et al 1977) Our findings on pancreatic TRF were confirmed by others using a boiling method for extraction, followed by gel filtration and assay (Morley et al. 1977), and by us with several thin layer and high pressure liquid chromatographic methods (Leppaluoto et al 1978) It is supposed the metabolic regulation of brain peptides involves peptidase degradation (Kuhl & Taubert 1975), and some of the cleavage products appear to be biologically active (Prasad et al. 1977) The purpose of this study was to obtain more information on tissue TRF by various degradation techniques and specially to investigate if the pancreatic TRF is degraded in the same way as brain TRF

MATERIAL AND METHODS

Extraction and determination of TRF Tissue samples were homogenized in methanol, evaporated washed with chloroform, purified by cation exchange chromatography and the TRF was measured by specific radioimmunoassay as described elsewhere (Ling et al 1976, Leppäluoto et al 1978).

Degradation of endogenous TRF in excised intact organs Excised hypothalami, extrahypothalamic brains and pancreata from decapitated rats were allowed to stand at room temperature (20°C), for 0 10 30 and 90 min before methanol extraction and determination of TRF The time for decapitation, excision and sample weighing was 1 3 min In another experiment rats were anesthetized with Nembutal (35 mg/kg) and the skull was opened from above With the animals anesthetized tissue samples were taken from the cortex and the thalamo-hypothalamic area and treated in two ways. Some samples were placed in liquid N within 10 and subsequently extracted in methanol. Secondly samples were taken as above but kept at room temperature for 3 min before they were immersed in N_2. TRF was determined as described

Degradation of exogenous and endogenous TRF in tissue homogenate 10 hypothalami extrahypothalamic brains and pancreata were crushed and homogenized in PBS (5 ml/g) in teflon-glass homogenizer Each tissue homogenate was divided into 2 equal parts and to one half synthetic TRF was added The homogenates were incubated at 37°C Samples were taken in cold methanol at 0, 5 10 and 30 min and TRF determined as described after chromatography

Sonication of tissue homogenates Tissue homogenates were produced as in the previous experiment. No TRF was added. Each homogenate was divided into 4 equal parts and sonicated (MSE, England. Settings: power low

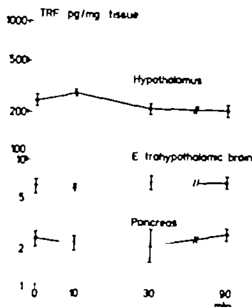


Fig 1 TRF content in excised intact organs at 22°C as a function of time

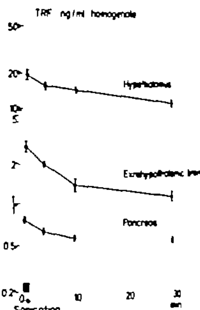


Fig 3 Endogenous TRF-concentration in incubated (37°C) tissue homogenates after sonication.

amplitude) for 1 min The sonicated homogenates were incubated at 37°C and samples were taken at 0 3 9 and 27 min after sonication

Degradation of synthetic TRF in rat serum Synthetic TRF (0 20 200 and 000 ng) was added to ml of diluted rat serum (1 1 PBS) and incubated at 37°C Aliquots were taken into cold methanol at various intervals for determination of TRF

Degradation of purified tissue TRF by rat serum Tissue homogenates were purified by cation exchange chromatography for TRF as described The lyophilized eluant was diluted in PBS (1 4 ml) and incubated with μl serum at 37°C Aliquots were taken as in the previous experiment.

All animals used were outbred Sprague Dawley rats. They were handled in the normal way All samples for one experiment were measured in the same assay. Mean values ± S E (*n*=4) are given if not otherwise indicated. The statistical significance was tested by Student's *t* test.

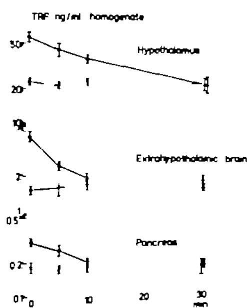
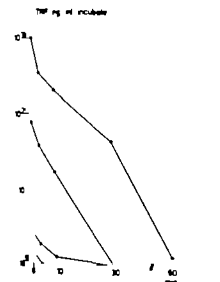


Fig 2 Concentration of TRF in incubated (37°C) tissue homogenates without (●—●) and with (O—O) synthetic TRF added

RESULTS

When excised intact samples of hypothalamus, extrahypothalamic brain and pancreas were allowed to stand at room temperature for up to 90 min there were no statistically significant losses of extractable TRF immunoreactivity (Fig 1) In the experiment with rapid sampling from anesthetized animals the thalamo-hypothalamic TRF concentration was 442±85 pg/mg in samples frozen within 10 s and 404±35 pg/mg in samples kept for 3 min at room temperature before processing The respective values for cortical brain were 0 51±0 20 and 0.69±0.21 pg/mg There were no statistically significant changes between values at 10 s and 3 min.

When synthetic TRF was added to tissue homogenates of hypothalamus extrahypothalamic brain and pancreas it was found to be degraded within 30 min whilst the endogenous TRF in these homogenates was not degraded in this time (Fig 2). Fig 3 shows that when homogenates were sonicated for 1 min there was a rapid decrease in TRF concentration during the first 10 min This degrad-



Degradation of various amounts of synthetic TRF ...ated ...th normal rat serum at 37°C. The means of ... are given

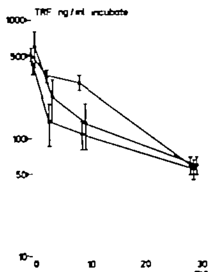


Fig. 5. Degradation of chromatographically purified tissue TRF when incubated with normal rat serum at 37°C. ●—● = hypothalamus, △—△ = pancreas, ○—○ = extrahypothalamic brain.

...of endogenous TRF in sonicated homogenates ...similar in character to that of synthetic TRF in ...t homogenates.

...the experiment where various amounts of ...etic TRF ...ere added to normal rat serum the ...activity was destroyed (Fig. 4). The TRF ...ity from purified hypothalamic, extrahypo...amic brain and pancreatic tissue extracts wa... ...aded by normal rat serum to about 1/10 of the ...al value in 30 min (Fig. 5).

...CUSSION

...purify methanol extracts by cation exchange ...matography because we have found that simple ...hanol extracts give higher TRF concentrations ...er RIA, especially if large amounts of tissue ...mg) are used. This is not due to a loss of TRF ...hromatography since the recovery of both syn...ic TRF and ...H-TRF in our chromatography is ...100%.

...fter the killing and excision procedures the tis... ...can be kept at room temperature with no loss of ...F immunoreactivity for up to 90 min. The ex...riment ...ith rapid sampling gave no proof of a ...ore rapid degradation immediately after death. If ...re are changes in the amount of TRF present they must occur within 10 s of excision. These results show that, with recent excision techniques degradation of endogenous TRF is not a problem.

The fact that exogenous, but not endogenous, TRF was rapidly destroyed in tissue homogenates indicates that endogenous TRF is well sheltered in the hypothalamus, extrahypothalamic brain and pancreas, all of which possess TRF degrading activity. Drastic manipulations such as sonication, alcohol extraction, osmotic shock, etc., have to be used to liberate TRF from its protected form. Parker et al. (1978) have demonstrated that most TRF in the hypothalamus is located in the soluble fraction of the synaptosomes and is easily obtained from osmotically lysed synaptosomes. The subcellular locus of TRF in the pancreas is not known. It is also possible that TRF does not occur as a tripeptide in the tissue.

It is well known that synthetic TRF is rapidly degraded by serum and various tissues. Similarly normal rat serum degraded the TRF immunoreactivity from purified hypothalamic, extrahypothalamic brain and pancreatic tissue extracts.

These degradation studies offer further evidence that the TRFs from pancreas and extrahypothalamic brain are identical with hypothalamic TRF and that in these tissues the TRF is protected in a similar way from an effective degrading

system Schaeffer et al (1977) showed that the release of TRF from hypothalamic synaptosomes is stimulated by K and dependent on Ca^{2} and that release can only be observed in the presence of a peptidase inhibitor It is possible that the physiologically active TRF which is rapidly degraded is only a small part of the total TRF Thus changes in TRF levels after stimuli to living animals are difficult to recognize We have elsewhere reported a rapid rise in hypothalamic TRF content after stressful stimuli to rats (Koivusalo & Leppäluoto 1979) which is best explained as being due to the inhibition of the TRF degrading system This together with the present findings indicates that the TRF degrading system is an important regulating mechanism in living animals

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HULTHÉN U L, DYMLING J F & HÖKFELT B Kinins in relation to kallikrein activity kininogen, electrolytes, aldosterone and catecholamines in urine from normal individuals. Acta Physiol Scand 1980, 110 307–314 Received 26 Feb. 1980 ISSN 0001-6772. Department of Endocrinology Lund University Clinics, General Hospital, Malmö Sweden.

The object of the present study was to test the hypothesis that urinary kinin excretion is an indicator of intrarenal kinin formation and to investigate urinary e cretion of kinins in relation to natriuresis, kaliuresis, diuresis and urinary aldosterone and catecholamines in normal individuals on free salt and water intake. In freshly voided urine collected from 4 normal individuals kinin concentration was directly related to kallikrein activity Kininogen concentration was very low and neither related to kallikrein activity nor to kinin concentration. The excretion rates of kinins and kininogen were unrelated t the time interval between micturitions In 24 hour urine collections from 50 normal individuals the excretion of kinins was positively correlated t natriuresis, kaliuresis and diuresis and also to dopamine but not to aldosterone noradrenaline and adrenaline Kinin xcretion was inversely related to age and was lower in women than in men On the basis of these results it is concluded that urinary kinin xcretion reflects intrarenal kinin formation in normal ambulatory individuals and that urinary kinins are formed mainly in the interstitial and vascular space of the kidney Furthermore, kinins and dopamine seem to play physiological role in the renal handling of electrolytes and water

Key ords Adrenaline, aldosterone diuresis, dopamine, kaliuresis, kallikrein, kininogen, kinin, ... natriuresis noradrenaline

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To test the hypotheses that urinary kinin excretion is an indicator of intrarenal kinin formation, kinin concentration, kallikrein activity and kininogen concentration were measured in urine from normal individuals.

Furthermore the interrelations between urinary kinin excretion and natriuresis kaliuresis and diuresis were studied As aldosterone sympathetic activity and renal dopamine are implicated in renal handling of electrolytes and water (Gordon et al 1965 Sealey et al 1972, Cuche et al. 197? Alexander et al. 1974) urinary excretion of aldosterone and catecholamines were also determined.

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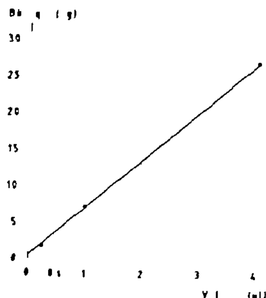


Fig 1 Kinin formation (Bk eqv) related to urinary volume 0 1 ml human plasma substrate and 15 min incubation time was used

was collected from 24 subjectively healthy individuals 8 men and 16 women aged ?0–62 years The time interval from the previous micturition was registered and urinary volume and pH recorded

For the determination of urinary excretion of kinins noradrenaline adrenaline dopamine aldosterone sodium potassium creatinine and water 24 hour urine was collected from 50 subjectively healthy individuals 1 men and ?9 women aged 23–7? years in a plastic container with 10 ml concentrated glacial acetic acid

Freshly voided urine was used in the first case because it was observed that acidification with glacial acetic acid gave an irreversible reduction of urinary kallikrein (kininogenolytic) activity Acidification of the urine was used to preserve kinins (see below) and catecholamines

METHODS

I Kallikrein activity

Urine was stored under toluene at +4°C until processed in a single assay run Urinary kallikre n acti ity was determined by incubating the urinary samples with human plasma substrate (HPS) and measuri g the amount of bradykinin equivalents (Bk eq) formed HPS was obtained from a normal individual by collecting blood in plastic tubes containing 0 2 mg hexadimethrine bromide (Polybrene Aldrich-Europe Belgium) 0 125 mg Soya Bean Trypsin Inhibitor (SBTI Sigma, St Louis, Mo) and 0 2 mg dipotassium-EDTA (Eastman Rochester NY) per ml blood and separating the plasma by centrifugation Polybrene was used to prevent plasma prekallikrein activation (Eisen 1964) and SBTI to inhibit activated plasma kallikrein (Werle & Maier 1952). Plasma collected in this way and stored at −20 °C showed a stable kininogen level for months as measured by incubation with trypsin (TPCK 228 U/mg 80% protein Worthington Biochem Corp Freehold NJ) or an excess of undiluted urine

The incubation procedure was a modification for Gr retero et al (1976) Urine was diluted 1/100 in Tris-H buffer 0 1 mol/l pH 8.5 containing dipotassium-EDTA 0 03 mol/l 1 10-phenanthroline monohydrochl (Fluka AG Switzerland) 0.01 mol/l and 8-hydroxy quinoline (Sigma) 0 001 mol/l. This buffer is referred t TBEPH 0.1 ml of diluted urine was incubated with 0.1 HPS in 0.8 ml TBEPH for 15 min at +37°C. 100 µl ... lyl-3 4 H(N)) bradykinin triacetate (New England Nu lear Boston Mass) was added as an internal standard the enzymatic reaction was terminated by the addition of ml ethanol 99.5% (v/). Kinins formed were measured radioimmunoassay (RIA) as described for bradykinin blood (Hulthén & Borge 1976) The antiserum bo bradykinin and lysylbradykinin (kallidin) equally ... but did not cross-react with the major inactive pept fragments of bradykinin Recovery of (³H) bradykinin 66 1±7 1% (mean ± S D). 0 1 ml HPS incubated TBEPH in the absence of urine corresponded to 0 4 g eqv This value was independent of incubation time was substracted from the values obtained with the test specimen

When incubating 5 ng Bk with 0.1–0.5 ml HPS TBEPH for 15 min no degradation of Bk was observ Increasing the urinary volume or the incubation time ga a linear increase in the amount of Bk eqv formed (Fig and respectively). With olumes of HPS from 0.1 0.5 ml maximal generation of Bk eqv occurred with 0.1 (Fig. 3) and this was a consistent finding using HPS fr several normal individuals. Adding 1.25 mg of SBTI stead of 0 125 mg per ml of blood for HPS did not decr the amount of Bk eqv formed with 0 1 ml HPS. In add less than 10% of the substrate was consumed as measur by simultaneous incubation of HPS with trypsin and excess of undiluted urine (4.2 µg Bk eqv/ml HPS for bot The intraassay coefficient of variation (cv) for ur kallikrein activity was 14 7%

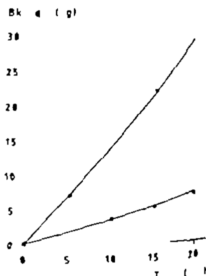


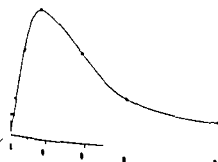
Fig 2 Kinin formation (Bk eqv) related to incubation times for two urinary samples 0 1 ml human plasma substrate and 1 µl urine was

... was directly prepared and incubated with trypsin in ... assay run according to Diniz & Carvalho (1963) ... (³H) bradykinin ... as added to the incubate as an ... standard and the enzymatic reaction was terminated by the addition of ethanol 99.5% ( ). Kinins were measured by RIA (Hulthén & Borge 1976). Recovery of (³H) bradykinin was 74.5±7.8%. The ... assay cv for urinary kininogen was 11.1%.

... samples were frozen until assayed. Urine was pre... and determined by RIA as described by Hulthén & ... (1976). Recovery of (³H) bradykinin, added as an ... standard, ... 78.9+5.2%. Serial dilutions of urinary preparations gave lines, which were parallel to the ... bradykinin standards in the RIA (Fig. 4). Generally ... corresponding to an original urinary volume ... were measured in each RIA tube.

... effect of acidification ... with glacial acetic acid (0.1 ml ... urine) on urinary kinin concentration was studied ... series of fresh samples from 4 individuals, which ... determined in ... single assay run. Kinin concentration was 36.6±18.9 ng Bk eqv/ml (mean ± S.D.) in non-acidified samples and 38.7 ... 18.5 ng Bk eqv/ml in acidified ... The difference was not significant. The cv between the two samples from each individual was 11.0%. ... correlation coefficient ... was 0.97 (*P*<0.001).

... stability of kinins in acidified urine was studied in ... series of fresh samples from 24 individuals which ... were determined in ... single assay run. Kinin concentration was 37.8±19.1 ng Bk eqv/ml in samples frozen ... after acidification and 39 ... 1.1 ng Bk eqv/ml in ... kept at room temperature for 4 h before they ... frozen. The difference was not significant. The cv ... the two samples from each individual was 9.5%. ... correlation coefficient 0.97 (*P*<0.001).
... interassay cv for urinary kinins was 12.0%.



... 3. Kinin formation (Bk eqv) related to volumes of ... plasma substrate (HPS). 1 μl urine and 15 min ... incubation time was used.

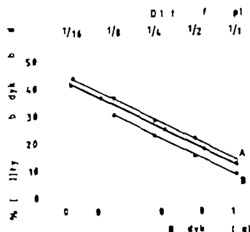


Fig. 4. Plot of percentage (¹²⁵I) tyrosin-bradykinin bound to antibody vs. log standard amount of bradykinin and log dilutions of prepared urinary samples respectively. Closed circles = standards of bradykinin. Open circles = dilutions of samples (A and B). Corresponding original urinary volumes were for A 80–5 μl and for B 20–...5 μl.

IV. *Noradrenaline, adrenaline and dopamine*

Free catecholamines in urine were selectively isolated on ... cation-exchange resin column especially developed for this purpose by Bio-Rad Laboratories, Richmond, Cal., and eluted with boric acid (Routh et al. 1971). Noradrenaline and adrenaline were measured spectrophotofluorometrically with the trihydroxyindole reaction (Euler & Floding 1965). Dopamine was quantified spectrophotofluorometrically after periodate oxidation (Anton & Sayre 1964, Routh et al. 1971). The interassay cv for noradrenaline, adrenaline and dopamine was 10.7, 13.5 and 10.8% respectively.

V. *Aldosterone*

Aldosterone in urine was determined by RIA after acid hydrolysis and extraction on ... Sephadex LH 20 column (Pharmacia, Sweden) (Ito et al. 1972). Interassay cv was 13.2%.

VI. *Urinary sodium, potassium and creatinine* were measured by standard laboratory techniques. *Urinary pH* was determined with ... glass electrode.

STATISTICAL EVALUATION

Statistical analyses was performed by non-parametric methods. Mann-Whitney ... rank-sum test was used to detect differences between men and women, and Spearman ... rank-correlation to calculate correlation coefficients ( ). The values are given as mean ± S.D. and the level of significance is taken as *P*<0.05.

Table 1 *24 hour urinary excretion of the variables studied (Mean ± S.D)*

	All subjects	Men (♂)	Women (♀)	Difference (♂ ♀)
n	50	1	79	
Age (y)	41±13	38±1	4_±14	n.s.
Kinins (μg Bk eqv/d)	4 .3±15.3	48.5±15 6	37.5±13.3	$P<0\ 01$
Aldosterone (nmol/d)	_5 6±1 _	77 3±13 4	24 3±11 1	n.s.
Dopamine (nmol/d)	1 624±551	1 884±605	1 436±427	$P<0\ 01$
Noradrenaline (nmol/d)	_51±100	77?±95	_35±102	n.s.
Adrenaline (nmol/d)	58±_5	73±77	48±16	$P<0\ 01$
Sodium (mmol/d)	147±60	168±64	131±53	$P<0.02$
Potassium (mmol/d)	6_± 1	70±__	55±2_	$P<0.02$
Creatinine (mmol/d)	13.5±4 1	17.3±3 2	10 7±1.5	$P<0\ 01$
Volume (l)	1.31±0 41	1.53±0 31	1 15±0 41	$P<0\ 01$

RESULTS

In freshly voided urine kallikrein activity was 8 1±7 7 μg Bk eqv/ml/15 min incubation kininogen concentration 10 3±6 1 ng Bk eqv/ml and kinin concentration 78 3±13 1 ng Bk eqv/ml Kinin concentration was positively correlated to kallikrein activity ($r=0\ 56$ $P<0\ 01$) but was neither related to kininogen concentration nor to pH (range 5.3–7.5) Kininogen concentration was not related to kallikrein activity but was negatively correlated to pH ($r=-0\ 67$ $P<0\ 01$) Neither kinin nor kininogen excretion (ng/min) was related to the time interval between micturitions (range 150–390 min)

In 24 hour urine the values for kinins dopamine adrenaline sodium potassium creatinine and volume were higher in men than in women (Table 1) Kinin excretion was positively correlated to the excretion of dopamine sodium potassium and creatinine as well as to urinary volume but was not related to the excretion of aldosterone noradrenaline and adrenaline (Table 2) There was an inverse relationship between kinin excretion and age Th values for dopamine noradrenaline and adrenalin were directly related to each other and show positive correlations to the excretion of sodium potassium as well as to urinary volume. Aldosterone excretion was directly related to noradrenaline excretion but did not correlate to natriuresis kaliuresis and diuresis

DISCUSSION

Most studies of the renal kallikrein-kinin system rest upon the determination of urinary kallikrein activity by esterolytic assays The specificity of these assays has recently been questioned (Geiger Mann & Bettels 1977 Levinsky et al 1979). Lack of specificity may explain some of the discrepant findings in earlier studies

The level of urinary kallikrein activity found in the present kininogenolytic assay is somewhat higher than that reported by Carretero et al (197

Table 2. *Interrelations between the variables studied (Spearman s rank correlation coefficients)* (n [illegible])

	U-Kinins	U Dopamine	U-Noradrenaline	U Adrenaline	U Aldosterone	U Na	U K	U-Creatinine	U-Volume	Age
U Kinins	×	0 45	0.20	0.19	0 ?7	0 48***	0.55	0 63	0.33	[illegible]
U Dopamine		×	0 4	0 54	0 11	0 59*	0 52	0 62	0.35*	[illegible]
U Noradrenaline			×	0 30*	0.33	0 31	0 41	0.35	0.34	[illegible]
U Adrenaline				×	0 2_	0.35	0 41	0 46	0.40*	[illegible]
U Aldosterone					×	−0 06	0 27	0 _3	−0 03	[illegible]
U-Na						×	0 62*	0.50*	0.46	[illegible]
U K							×	0.55**	0.37*	[illegible]
U-Creatinine								×	0 90*	[illegible]
U Volume									×	[illegible]
Age										

$P<0\ 001$ $P<0\ 01$ $P<0\ 05$

sible explanation is that Carretero et al. (1976) partly purified kininogen from dog plasma as rate. The decrease in Bk eqv formed when using the volume of HPS beyond 0.1 ml is in accordance with the finding of Marin-Grez & Carretero (1972) using heated dog plasma as substrate. Likely it is due to kallikrein inhibitory activity in plasma substrate (McConnell 1972).

Stop-flow studies in the dog indicate that renal kallikrein is secreted into the urine in the distal part of the nephron (Scicli et al. 1976) and that kinins appear in the urine at this level (Scicli, Gandolfi & Carretero 1978). When injecting bradykinin into the renal artery in dogs, urinary kinin excretion did increase (Abe 1965). The present finding of a positive correlation between kallikrein activity and kinin concentration in fresh urine supports the concept that both these variables reflect the activity of the renal kallikrein-kinin system in normal individuals on a free salt and water intake. Abe et al. also found a direct relation between urinary excretion of kallikrein and kinins in samples from normal individuals obtained before and following an intravenous injection of furosemide.

The origin of urinary kininogen has not been established. The concentration presently found is less than 1% of the kininogen level in human plasma (Jacobsen & Sepila 1971). Similar values were reported by Pisano et al. (1978). The lack of correlation between kininogen concentration and kallikrein activity and between kininogen excretion and the time interval between micturitions indicates that the low kininogen level is not primarily due to the action of kallikrein in the urinary tract. The indirect relationship between kininogen concentration and urinary pH supports some pH-dependent formation of kinins in the urinary tract but kinin concentration did not correlate to urinary pH. Accordingly the main part of urinary kinins seems not to be formed in the urine.

Renal kallikrein is present in the interstitial and tubular space of the kidney (de Bono & Mills 1974, Carretero et al. 1976), where the conditions for kinin generation are more favourable than in the urine with respect to kininogen level (Werle & Zack 1970, Jacobsen & Sepila 1971) and pH (Geiger, Mann & ... 1977). Kinins formed in this location may enter the urine at the level of the distal tubule where the capacity for enzymatic cleavage of kinins is very limited (Carone et al. 1976).

The lack of correlation between urinary kinin excretion and the time interval between micturitions supports the concept that degradation of kinins in the lower urinary tract is of minor importance and that urinary kinin excretion is a good indicator of intrarenal kinin formation. Furthermore kinins are stable at room temperature for 24 h in urine acidified with glacial acetic acid and the generation of methionyllysylbradykinin by uropepsin (Hial, Keiser & Pisano 1976) appears to be minimal under these conditions. The level of kinin excretion in 24 hour urine found in this study is very similar to that reported by other investigators using radioimmunoassay (Mashford & Roberts 1971, Shimamoto et al. 1978) or bioassay (Jensen, Venneröd & Rinvik 1965, Mashford & Roberts 1971). Lower values have however also been reported (Abe et al. 1965, Hial, Keiser & Pisano 1976, Vinci et al. 1978). The reason for these differences is not clear.

The finding of positive correlations between urinary kinin excretion and natriuresis, kaliuresis and diuresis is compatible with the concept that kinins play a physiological role in renal handling of electrolytes and water. This hypothesis was presented by Adetuyibi & Mills (1972) who found a positive correlation between urinary excretion of kallikrein and sodium in normal subjects on a free salt intake and an increase in urinary kallikrein excretion after a water load. Direct relations between urinary kallikrein excretion and natriuresis and diuresis have been found in normal subjects by Abe et al. (1978) and Halushka et al. (1979) but not by Margolius et al. (1974). Abe et al. (1978) also found that urinary kinin excretion was positively correlated to natriuresis and diuresis.

It has, however, been reported that urinary kallikrein excretion increased during sodium depletion in normal man (Margolius et al. 1974, Vinci et al. 1979). This increase was inhibited by spironolactone and is thus most likely due to enhanced aldosterone secretion (Margolius et al. 1974). Increased urinary kallikrein excretion has also been found in primary aldosteronism and in Bartter's syndrome (Margolius et al. 1971, Vinci et al. 1978). In these conditions urinary kinin excretion was, however, low-normal (Abe 1965, Vinci et al. 1978, Hulthén et al. 1977) suggesting that the increase in urinary kallikrein excretion induced by aldosterone does not reflect enhanced intrarenal kinin generation. This is compatible with the report of Vinci et al. (1979) who found a decrease in urinary aldo-

sterone and kallikrein excretion in normal subjects changing from very low to high sodium intake which was not accompanied by any alteration in urinary kinin excretion

Vinci et al (1979) observed no differences with regard to urinary excretion of kallikrein and kinins in normal subjects equilibrated on normal as compared to high sodium intake All individuals were however on a fixed fluid intake of ? 5 l/day Experimental data indicate that augmented sodium intake stimulates the renal kallikrein kinin system only when fluid intake could increase to allow the expansion of some critical fluid volume possibly the extracellular fluid volume (Marin-Grez & Carretero 1973 Mills & Ward 1975) The fixed fluid regime may thus be the reason for the lack of differences in urinary excretion of kallikrein and kinins on normal as compared to high sodium intake in this study

The present finding of positive correlations between urinary kinins and urinary potassium and creatinine seems consistent with data in the epidemiological study by Zinner et al (1976) On the other hand Vinci et al (1979) found that a change from high to low potassium intake in normal subjects was associated with a decrease in urinary kallikrein excretion but no change in urinary kinin excretion As the decrease in urinary kallikrein excretion was closely related to a reduction in aldosterone excretion it probably did not reflect diminished intrarenal kinin generation

The lack of a direct correlation between urinary aldosterone and urinary kinins in the present study supports the concept that aldosterone does not increase intrarenal kinin formation The lack of significant correlations between urinary aldosterone and natriuresis and kaliuresis is in accordance with the findings of Tolhagen & Karlberg (1978) in 1?0 ambulatory normal individuals The positive correlation observed between urinary noradrenaline and aldosterone suggests that aldosterone secretion is to some extent under the influence of the sympathetic nervous system probably mediated by an increase in renin release (Gordon et al 1967)

Whereas urinary noradrenaline and adrenaline mainly reflect the general sympathetic activity urinary dopamine is chiefly of renal origin (v Euler Floding & Lishajko 1959 Alexander et al 1974) When given i v dopamine increases urinary sodium excretion (McDonald et al 1964) and previous studies have shown a direct relationship between urinary dopamine and natriuresis (Cuche et al 1972 Ball & Lee 1977) This was confirmed in the present study Furthermore there was a positive correlation between urinary kinins and dopamine A jointly vasodilatory and natriuretic action of dopamine and kinins in the kidney has been suggested (Kuchel Buu & Unger 1970). When infused into the renal artery of dogs dopamine increased urinary kallikrein excretion in association with a natriuresis (Mills & Obika 1976) The direct correlations between urinary dopamine and urinary noradrenaline and adrenaline presently found indicate that renal dopamine release is positively influenced by the sympathetic activity in normal ambulatory individuals on a free salt and water intake

A positive correlation between urinary noradrenaline and sodium may seem contradictory to earlier reports of lower urinary noradrenaline on high as compared to very low sodium intake (Gordon et al 1967 Alexander et al 1974) On the other hand Cuche et al (197?) did not find a decrease in urinary noradrenaline or adrenaline on very low as compared to normal sodium intake Mills & Obika (1977) reported that infusion of noradrenaline into the renal artery of the dog in a non-vasoconstricting dose induced a small increase in urinary excretion of sodium potassium and water It is possible that a direct relationship between urinary noradrenaline and natriuresis is lost in sodium depletion due to increase in sympathetic activity by a decline in plasma volume (Kelsch et al 1971)

The inverse relationship observed between urinary kinin excretion and age is consistent with the early finding of Elliot & Nuzum (1934) of decreasing urinary kallikrein excretion with age in 50 subjects with normal blood pressure

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·ntral interaction between reflex responses ·i activity in aortic nerve A and C-fibres

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The question of whether or not activity in myelinated (A) and non-myelinated (C) afferents in baroreceptor nerve show interaction of their refle effects, was investigated in 10 anesthetized rabbits. The central end of the cut left aortic nerve was stimulated by tw sets of electrodes, one for selective high-frequency excitation of A-fibres (A-stimulation) and one for low-frequency activation of C (and A) fibres by another pulse generator (C-stimulation) The pulse rates in C-stimulations were too low to evoke reflex effects via A-fibres. The hypotensive response to combined stimulations of A- and C-fibres (AC-stimulation) was found to exceed the sum of responses to separate A and C-stimulations in 21 of 22 stimulation series. For sympathetic activity to the kidney greater than additive effect as observed in 16 of 24 series ($P=0.06$) while in 6 of the series, the responses were equal Median values of the ratio AC/(A C) were 1.28 for the reflex changes in pressure and 1.11 for the effects on renal nerve activity Similarly C-stimulation which in comparison to A-stimulation affected sympathetic activity relatively more than blood pressure in 13 of 16 series ($P=0.01$) produced a greater sympathetic inhibition when added to background of A-fibre activity than when alone. These results suggest that ynergistic interaction xists between central effects of afferent discharge in aortic nerve A- and C-fibres.

Key ords Aortic nerve electrical stimulation, myelinated and non-myelinated afferents baroreceptor reflex, arterial pressure sympathetic activity

·cent work on the response characteristics of ·roreceptor C-fibres in rabbits and rats has shown ·at the C-fibre receptors have a higher pressure ·reshold than the A-fibre receptors and a lower ·scharge frequency (Jones & Thorén 1977 Thorén ·Jones 1977 Thorén, Saum & Brown 1977) In the ·bbit, they contribute to the baroreflex response to ·an abrupt rise in pressure only when mean pressure ·increased by more than 20 mmHg from normal ·ting pressures, to levels above 110 mmHg (Aars, ·Tyre & Haswell 1978)

The present study is an attempt to examine ·nother aspect of A and C-fibre participation in the ·aroreflex: the question of whether reflex effects of ·ctivity in the two types of fibres are subject to ·imple addition, or whether a synergistic or inhibi·tory central interaction exists between the two. To this end, I compared the reflex responses to electrical stimulation of aortic nerve A or C-fibres to that of A and C-fibres combined, in anesthetized rabbits As parameters of the reflex I used changes in arterial blood pressure and sympathetic activity to the kidney

METHODS

The experiments were performed in 10 rabbits, of body weights 3.0–3.9 kg For anesthesia, they received an injection of Hypnorm (AB Mekos, 0.10–0.15 ml/kg), followed by mixture of chloralose (0.03 g/kg) and urethane (0.75 g/kg) half and half p Urethane was supplemented when necessary The animals were tracheotomized and respired air Blood pressure was measured with transducer (Statham P23Dc) connected to catheter in the left femoral artery Efferent sympathetic activity was recorded in the left renal nerve by means



Fig. 1. Reflex changes in average renal nerve activity and mean blood pressure (AP) in response to separate and combined stimulation of A and C fibres in the left aortic nerve. *A*: A fibre stimulation with pulses of 0.0. ms, 6 V, 60 Hz in heart synchronous trains of 80 ms duration. *C*: Effects via C fibres during continuous stimulation with pulses of 1 ms, 6 V, 4 Hz. *AC*: The same stimulation characteristics as in A and C but applied simultaneously. *C on A*: C stimulation started 30 s after onset of A stimulation. For both reflex parameters AC>(A+C) responses and C on A >C.

of sil er electrodes and a differential amplifier (Grass P15). The nerve wa cut d tally. The activity was averaged (Coupler 985-A) and recorded on a Beckman R 411 Dynograph together with pulsatile and mean arterial pressure and heart rate. Output from the averager was linearly related to frequency of input signal within the range of activity observed in the renal nerve. The heart rate was obtained from a tachometer (Coupler 9857B) which was triggered through a pecial circuit by the ri ing phase of the arterial pressure. The same circuit could also be used to trigger the pulse generators (Grass SD9 and S48) used for stimulation of the left aortic nerve. This nerve was cut low in the neck and placed on two pairs of silver electrodes, one for A fibre stimulation (pulses of 6 V, 0.0 m duration and 60–100 Hz in trains of 80–100 ms duration), the other for stimulation of C-fibres (6 V, 1 ms, –4 Hz continuous stimulation or in two animals, one pulse every second or fifth heart beat triggered with a fixed 5 ms delay).

Stimulat on periods were 10 or 20 s with intervals of about 1 min. In each period one of the following types of stimulation was applied: (1) A-fibre timulation, high frequency (marked A); ( ) C fibre timulation, low frequency (marked C); (3) A and C fibre timulation combined (marked AC); (4) C-fibre stimulation added 10–45 after start of A fibre stimulation (marked C on A). The stimulations were usually repeated once or twice in variable sequence in order to obtain average responses. Complete series consisting of these 4 types of stimulation were run for several different stimulation characteri tics in each animal.

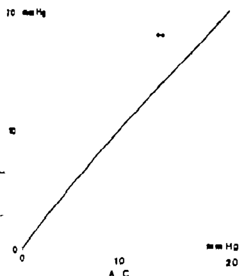
The pulse duration used for A stimulation wa below threshold for excitation of C-fibres, but during C timulation A-fibres were also activated. Classification of reflex effects as due to separate activation of A and C-fibres n A and C stimulation respecti ely i based on the fact that at the low frequency used in C stimulation A-fibre discharge is devoid of reflex act ons (Dougla, Ritchie & Schaumann 1956, Kardon, Peterson & Bishop 1973, Akre & Aars 1977, Aars, Myhre & Haswell 1978).

The results were read as maximum reductions in blood pressure and sympathetic activity (mm on recording paper) and mean value were usually compared [...] or as the ratio AC/(A+C). For comparison of [...] changes evoked by A and C-fibre activation, the [...] tions in blood pressure and sympathetic discharge [...] expressed in per cent of the immediately preceding [...] activity. Zero renal nerve activity was determined [...] level of the electrical noise recorded at the end of [...] ment after the nerve had been cut cranially but [...] in contact with the ti sues.

Experiment included in this presentation were [...] to those in which (*a*) control blood pressure were [...] mmHg mean, 60 mmHg diast lic, (*b*) the sum of [...] responses in each series wa less than 25 mmHg and [...] reduction in renal nerve acti ity and the summed [...] sure responses did not reach mean pressure levels [...] 50 mmHg, (*c*) responses remained unaltered through[...] the expt.

RESULTS

Reflex responses to combined stimulation of A [...] C fibre (AC) usually exceeded the summed [...] sponses to separate A and C fibre stimulation. Th[...] is shown for a typical experiment in Fig. 1 and [...] the whole material in Figs. 2 and 3. For the [...] pres ures AC>(A+C) was found in 1 of 2 [...] lation series ($P<0.01$); equivalent figures for [...] reduction in renal nerve activity were 16 of [...] ($P=0.08$). Average ratios of AC/(A+C) (median [...] 25–75 percentiles) were 1.28 (1.08–1.52) and 1.[...] (1.00–1.20) respectively. Intra-individual var[...] tions in reflex responses were small as shown [...] observations in three animals, in which mean [...] sponses were based on 3–4 consecutive A, C a[...] AC stimulations. In these series individual rea[...] ings differed from the calculated mean values [...] less than 2 mm for sympathetic and 3 mmHg [...] pressure responses.



2. Reflex reductions in blood pressure. A comparison line of identity between responses to combined stimulation (ordinate) and the sum of responses to separate stimulation (abscissa) of aortic nerve A- and C-fibres. Various stimulation characteristics 22 stimulation ... five rabbits Cf. Methods for further details.

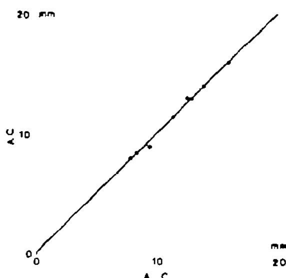


Fig 3 Reflex inhibition of renal nerve activity (read as millimeter on recording paper). A comparison between effects of combined and separate stimulations of aortic nerve A- and C-fibres, plotted as in Fig 2. 4 observations in 6 rabbits.

Reflex effects of "C on A -stimulations were compared to the effects of C and AC-stimulations ... series in 9 rabbits As shown by the series in Fig. 1 the sympathetic inhibitory effect of A stimulation tended to decrease with time whereas the pressive response usually increased before the set of C-stimulation. This difference was re... sted in the comparison of effects of C on A ... AC-stimulations: The sums of the A-effects ... at the start of C stimulation and the ... bed C-effects were invariably smaller than the C-responses when sympathetic activity was measured, and larger when calculated for the hypotensive responses. However C-stimulations were ... more effective when added to A-stimulation than when alone Thus, in 16 series, the reduction in renal nerve activity was increased in 13 ($P = 0.01$) unaltered in two and reduced in one when C-stimulation was superimposed on A-fibre discharge. For reflex changes in blood pressure these figures were 7 6 and 3 respectively The ratio "C on A /C was 1 19 (median, 25–75 percentiles 1 10–1.26) for the sympathetic responses. It was not calculated for the effects on pressure since the responses of this parameter to C-stimulation were rather small (Fig. 5).

While excitation of A fibres by C-stimulation did not disturb the study of reflex effects of separate A and C-stimulations (cf Methods) the "extra" A fibre activation during AC stimulation might coincide with the trains of A stimulation, thereby causing increased A-fibre discharge in a frequency range *above* threshold for reflex actions. Since the reflex effects of AC-stimulation surpassed the (A+C)-effects (Figs 1–3) it was necessary to evaluate how much of the increase might have been due to the extra A-fibre activity A typical example of this evaluation is shown in Fig 4 in which the sympathetic reduction resulting from three series of A C and AC stimulations have been plotted together with the calculated influence of the extra A fibre activity A-stimulation was performed by heart synchronous trains of 80 Hz/80 ms duration, 80/100 or 100/100, equivalent to respectively 6 8 and 10 pulses per heart beat. With a heart rate of 320 beats/min, the constant 2 Hz C timulation will hit less than two A trains per second, equal to an average increase of about 0.3 A-impulses per heart beat From the recorded responses to A-stimulation alone (Fig. 4) this addition can be expected to produce a further 1 2% reduction in sympathetic activity As shown in this figure and supported



Fig 1 Reflex changes in average renal nerve activity and mean blood pressure (AP) in response to separate and combined stimulation of A and C-fibres in the left aortic nerve *A* A fibre stimulation with pulses of 0 05 ms 6 V 60 Hz in heart-synchronous trains of 80 ms duration *C* Effects via C fibres, during continuous stimulation with pulses of 1 ms 6 V 4 Hz *AC* The same stimulation characteristics as in A and C but applied simultaneously *C on A* C stimulation started 30 s after onset of A stimulation. For both reflex parameters AC>(A+C) responses and C on A >C

of silver electrodes and a differential amplifier (Grass P15) The nerve was cut distally The activity was averaged (Coupler 9852A) and recorded on a Beckman R 411 Dynograph together with pulsatile and mean arterial pressure and heart rate Output from the averager was linearly related to frequency of input signal within the range of activity observed in the renal nerve The heart rate was obtained from a tachometer (Coupler 9857B) which was triggered through a special circuit, by the rising phase of the arterial pressure The same circuit could also be used to trigger the pulse generators (Grass SD9 and S48) used for stimulation of the left aortic nerve This nerve was cut low in the neck and placed on two pairs of silver electrodes one for A fibre stimulation (pulses of 6 V 0 05 ms duration and 60–100 Hz in trains of 80–100 ms duration) the other for stimulation of C-fibres (6 V 1 ms 4 Hz continuous stimulation or in two animals, one pulse every second or fifth heart beat triggered with a fixed 5 ms delay)

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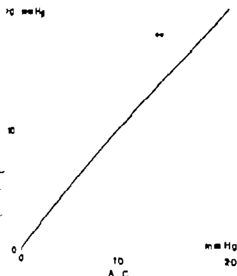
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2. Reflex reductions in blood pressure. A comparison, with line of identity, between responses to combined stimulation (ordinate) and the sum of responses to separate stimulation (abscissa) of aortic nerve A- and C-fibres, various stimulation characteristics. 22 stimulations in five rabbits. Cf. Methods for further details.

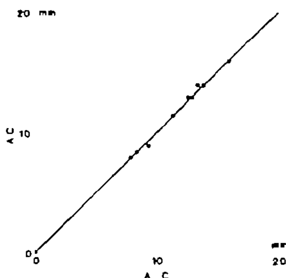


Fig. 3. Reflex inhibition of renal nerve activity (read as millimeter on recording paper). A comparison between effects of combined and separate stimulations of aortic nerve A- and C-fibres, plotted as in Fig. 2. 24 observations in 6 rabbits.

Reflex effects of "C on A" stimulations were compared to the effects of C and AC-stimulations in 16 series in 9 rabbits. As shown by the series in Fig. 1 the sympathetic inhibitory effect of A-stimulation tended to decrease with time whereas the depressive response usually increased before the onset of C-stimulation. This difference was reflected in the comparison of effects of "C on A" and AC-stimulations. The sums of the A-effects occurring at the start of C-stimulation and the isolated C-effects were invariably smaller than the AC-responses when sympathetic activity was measured, and larger when calculated for the hypotensive responses. However C-stimulations were usually more effective when added to A-stimulation than when alone. Thus, in 16 series, the reduction in renal nerve activity was increased in 13 (P<0.01), unaltered in two and reduced in one when C-stimulation was superimposed on A-fibre discharge. For reflex changes in blood pressure the figures were 7, 6 and 3 respectively. The ratio "C on A"/C was 1.19 (median, 25–75 percentiles 1.10–1.26) for the sympathetic responses. It was not calculated for the effects on pressure since the responses of this parameter to C-stimulation were rather small (Fig. 5).

While excitation of A-fibres by C-stimulation did not disturb the study of reflex effects of separate A- and C-stimulations (cf. Methods) the "extra" A-fibre activation during AC stimulation might coincide with the trains of A stimulation, thereby causing increased A-fibre discharge in a frequency range *above* threshold for reflex actions. Since the reflex effects of AC-stimulation surpassed the (A+C)-effects (Figs. 1–3) it was necessary to evaluate how much of the increase might have been due to the "extra" A-fibre activity. A typical example of this evaluation is shown in Fig. 4, in which the sympathetic reduction resulting from three series of A, C and AC-stimulations have been plotted together with the calculated influence of the "extra" A-fibre activity. A-stimulation was performed by heart synchronous trains of 80 Hz/80 ms duration, 80/100 or 100/100, equivalent to respectively 6, 8 and 10 pulses per heart beat. With a heart rate of 320 beats/min, the constant 2 Hz C-stimulation will hit less than two A trains per second, equal to an average increase of about 0.3 A-impulses per heart beat. From the recorded responses to A-stimulation alone (Fig. 4), this addition can be expected to produce a further 1.2% reduction in sympathetic activity. As shown in this figure and supported

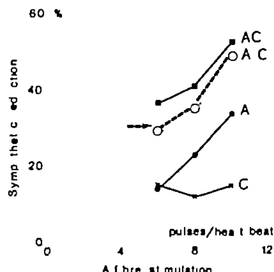


Fig 4 Plot showing the calculated sympathetic effect of "extra A fibre activity induced by the C stimulation of AC stimulations. Three stimulation series with different A fibre characteristics and a constant Hz C-stimulation (abscissa valid only for A stimulations) Stimulation periods of 10 s each See text for further description. In all three series the observed response to combined stimulation (*AC*) exceeded the sum of responses to separate A and C stimulations plus the calculated extra A-effect (arrow)

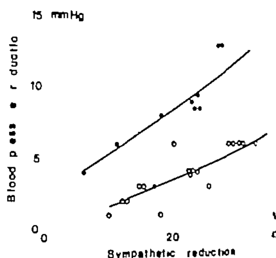


Fig 5 A comparison between reductions in arterial blood pressure and sympathetic activity to the kidney during stimulation of aortic nerve C-fibres (open circles) at low frequencies and A-fibres at high frequencies (dots) Regression equations to the lines are respectively y= 0 18x-0.2 r=0 76 and y=0.30x+2 4 r=0 65 Same stimulation series as in Fig.

by similar observations in all other animals subjected to more than one type of A stimulation the addition to (A+C)-effects of the calculated effects of the A fibre discharge caused by C stimulation was insufficient to explain the larger responses to AC than to (A+C)- stimulations

Heart rate responses to stimulation were small always less than 10 beats/min and frequently absent However in 23 series where at least one of the responses exceeded two beats/min AC-effects were larger than (A+C) in all but two Resting heart rates ranged from 230 to 320 beats/min

The experiments allowed a comparison to be made between reflex responses to A and C-fibre stimulation Relatively to the inhibition of renal nerve activity the effect on blood pressure was larger during A than C stimulation (Fig 5)

DISCUSSION

In a study of interaction between central effects of electrically evoked activity in A and C-fibres of the same aortic nerve it is obviously essential that the afferent discharge induced by combined AC stimulation does not differ from the sum of separate A and C stimulations This was not directly controlled in the present series, but the use of low-frequency stimulation of C-fibres makes it unlikely that collision between the simultaneously evoked action potentials in A fibres and the high-frequency potentials induced by the train stimulation of A-fibres should have much influenced the results If anything, collision would have *reduced* the number of evoked potentials during combined- as compared to the sum of separate stimulation

A more complex question is whether the excitation of A fibres by C stimulation the extra A fibre activity exerted different central effects during AC than during C stimulations The 2-4 Hz C stimulation equivalent to about 0 4-1 pulse/heart beat was undoubtedly below threshold for eliciting reflex effects by action potentials in A-fibres (Fig 4) However if during AC stimulation one or more of these A fibre activations coincided in time with the train stimulation of the same fibres, they presumably would add to the reflex effects of the other impulses in the train There are two reasons for believing that the synergistic interaction demonstrated in Figs 1-3 is *not* due to this type of artifact First because the estimated effects of A fibre activity superimposed on the trains of A-fibre discharge invariably failed to explain the larger responses to AC than to (A+C)-stimulations (Fig 4) Secondly because alterations in delay between start of A trains and C sti ul tion from 50 to 1

did not affect the AC/(A+C) ratio. This was y tried in one animal which unfortunately had to excluded from the material due to too severe spathetic responses to stimulation (cf Methods) The final prerequisite for the present study is that evoked afferent discharge did not exceed the ge of linearity of the reflex. To meet this re rement, I tentatively set the upper limits of re nses at 60% reduction in renal nerve activity 25 mmHg drop in blood pressure (cf thods). While the linearity was not directly ted up to these values, the results in Figs. 2 and 3 ell as a predominance of inhibitory interactions en they occasionally were exceeded (excluded eriments), probably constitute the best evidence t the limits were adequate for most animals. ever the level of background sympathetic ac ity i.e., activity not controlled by input from the aortic nerve will vary from one animal to other If particularly high, this background could ly push the linear range of reflex control via the tic nerve to less than 60% of renal nerve activi and hence make the response to combined mulation smaller than it would have been in a ter system. For this reason and because of the ssibility of collision between A-fibre impulses oked by the two pulse generators, I feel that the thods employed in this study have underesti ted rather than overestimated the synergistic re l interaction between activity in aortic nerve A- d C-fibres.

Results of the "C on A -stimulations cannot be mpared to AC-stimulations due to variations in amount of reflex effects of the A-stimulation maining at the start of C stimulation Time and ssure-related influences from other baroreceptor eas are obviously important in this context, mak g the evaluation of these series more difficult than e AC vs. (A+C)-series. Comparison of responses "C on A and C-stimulations did show how ver that the C-fibre discharge caused greater re ion changes in renal nerve activity and to some tent in arterial pressure, when the A-fibres were ready activated. Thus, the synergistic interaction etween central effects of discharge in A and C fibre afferent could be observed during two differ ent modes of stimulation as well as by two different reflex parameters, arterial blood pressure and sym- pathetic activity to the kidney

Previous investigations of reflex responses to specific stimulation of aortic nerve afferents have mostly been limited to comparisons of effects of activity in myelinated vs all fibres (Douglas, Ritchie & Schaumann 1956, Kardon Peterson & Bishop 1973) Such studies have shown that activity in C-fibres will induce reductions in heart rate and blood pressure at discharge rates below threshold for elicitation of reflexes via A-fibres Kardon Peterson & Bishop (1975) also managed to study the effects of C vs. AC-stimulation by means of anodal block of the evoked A-fibre activity Judged by the reflex changes in heart rate activity in A and C fibres was found to be about equipotent in the rabbits. It should be noted however that the fibres were stimulated at the same and rather high fre quencies, far in excess of the discharge rate normally occurring in the C-fibres of the rabbit's aortic nerve (Thorén & Jones 1977) For this reason and as their experiments were not designed to evaluate the effects of combined vs separate stimulation of A and C-fibres, their results are not relevant to the present series of expts.

The question of summation of baroreceptor input from different nerves has repeatedly been studied but always without separation of effects via A and C-fibres. In the most recent paper in this category Kendrick, Matson & Lalley (1979) reported that, in the dog, reflex changes in blood pressure heart rate and vascular resistance in leg muscles in response to combined ipsilateral stimulation of the aortic and carotid sinus nerves exceeded the sum of separate stimulations This illustration of synergistic interac tion between reflexes from baroreceptor areas was performed by stimulation of mainly large afferents and the specific role of A and C fibres was left open in the discussion. The present investigation adds to our knowledge on this point by showing that synergistic interaction may exist also between re flex responses to activity in myelinated and non-myelinated fibres within the same baroreceptor nerve and that the refle actions of activity in one type (C) can be potentiated by simultaneous activity in the other The latter finding is highly relevant to the normal function of these fibres, since the pres- sure needed to activate receptors with C-fibres is well above the threshold for evoking A-fibre discharge in the same nerve.

Finally it is worth noting that, relatively to the reflex changes in blood pressure C fibre activity evoked a stronger inhibition than A-fibre activity of the sympathetic discharge to the kidney (Fig. 5) Since heart rate changes were minimal (probably

due to anesthesia) the changes in blood pressue reflected the influence on sympathetic vasocon strictor activity in general Stronger effects of C than A fibre activity on renal nerve activity at equal pressure effects accordingly means that the reflex responses to aortic nerve A fibre activity must have surpassed the effects mediated by C fibre projections to sympathetic neurons serving other peripheral vascular beds This deduction fits the observed responses to aortic nerve A and C fibre stimulation in cats relatively to blood pressure changes A fibre activity produced a greater reduc tion in vascular resistance of calf muscle than did the C fibre activity (Öberg et al personal communi cation) Taken together these observations illus trate interesting and important aspects of differen tiated cardiovascular control

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Evidence for differential localization of angiotensin I converting enzyme and renin in the corpus striatum of rat

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Evidence for the presence of the components of a renin-angiotensin system in brain has been published (Ganten & Speck 1978, Phillips et al 1979). In particular the enzyme renin (E.C 3 4 99 19) has been purified from brain tissue of several species including rat (Hirose et al. 1978), man (Ganten & Speck 1978) and mouse (Speck & Ganten in preparation). Brain renin was found to be distinct from cathepsin D-like acid proteases (Hirose et al 1978, Speck & Ganten in preparation, Osman et al. 1979, Ruyschak et al. 1978, Ganten & Speck 1978). The enzyme is immunologically similar to kidney and submaxillary gland renin.

The peptidyl dipeptide carboxyhydrolase (E.C 3.4.15 1) which converts the decapeptide angiotensin-I (ANG I) to the effector octapeptide angiotensin-II (ANG II) has also been found to be present in brain (for review see Ganten & Speck 1978, Phillips et al. 1979). Angiotensin-II-like immunoreactivity has been demonstrated within certain types of nerve cells within the brain and spinal cord of the rat (Fuxe et al. 1976, Ganten et al. 1978) and specific angiotensin-II receptors seem to exist within the mammalian brain (Bennett & Snyder 1976, Sirett et al 1977). Furthermore, a selective depletion of activity of angiotensin-I converting enzyme (E.C 3 4 15 1) has been demonstrated in the corpus striatum of patients with Huntington's chorea (Arregui et al. 1977).

In the present paper evidence has been obtained for a neuronal location of angiotensin-I converting enzyme using ibotenic acid induced lesions of the corpus striatum (see Schwarcz et al. 1979). Ibotenic acid, like kainic acid, produces a selective degeneration of the nerve cells within the injected area without damaging afferent nerve terminals. The local neurotoxic action of ibotenic acid does not cause distant lesions in other parts of the brain as is the case with kainic acid (Schwarcz et al. 1979).

Male specific pathogen-free Sprague Dawley rats were used (150–200 g b.w.). The right corpus striatum was lesioned by means of 4 stereotaxic injections with ibotenic acid as described by Fuxe et al. (1979). In some animals the extent of the lesion was studied by routine histological analysis using Nissl staining such as toluidine blue or thionine. The activity of angiotensin-I converting enzyme, renin and cathepsin D-like acid protease was measured. The procedures and criteria of specificity for differential measurement of renin and cathepsin D-like acid protease have previously been published (Ganten & Speck 1978, Depierre & Roth 1975). For converting enzyme measurements N carbobenzoxy L-phenyl-alanine L-histidine-L-leucine has been used.

Renin activity was expressed as picomoles angiotensin-I formed/mg protein/1 h. Cathepsin D-like acid protease activity was expressed in μg BSA equivalents/mg protein/h and specific angiotensin-I converting enzyme activity was expressed in picomoles of histidine-leucine/mg protein/min. The rats were killed 3 weeks after operation by rapid decapitation and the lesioned and unlesioned corpus striatum was dissected out and frozen for subsequent enzyme analysis.

The results are summarized in Table I. The angiotensin-I converting enzyme activity is reduced by about 50 % on the operated side compared with the unoperated side. However, the ibotenic acid induced degeneration of nerve cells in the corpus striatum produces an increase in renin and cathepsin D-like acid protease activity by about 50 % on the lesioned side compared with the unoperated side (Table I). A histological analysis showed an

due to anesthesia) the changes in blood pressure reflected the influence on sympathetic vasoconstrictor activity in general Stronger effects of C than A fibre activity on renal nerve activity at equal pressure effects accordingly means that the *reflex responses to aortic nerve A fibre activity* must have surpassed the effects mediated by C fibre projections to sympathetic neurons serving other *peripheral vascular beds This deduction fits the* observed responses to aortic nerve A and C fibre stimulation in cats relatively to blood pressure *changes A fibre activity produced a greater reduc*tion in vascular resistance of calf muscle than did the C fibre activity (Öberg et al personal communi*cation) Taken together these observations illus*trate interesting and important aspects of differentiated cardiovascular control

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Table 1 *The effects of ibotenic acid induced lesions of the corpus striatum of normal male rats on angiotensin I converting enzyme activity renin enzyme activity and cathepsin D-like acid protease activity*

For details on the operation procedure see text Means ± S E are shown out of 10-11 rats. Student's t-test was used for the statistical analysis

	Lesioned striatum	Statistical difference Lesioned vs. intact striatum	Unlesioned striatum
Converting enzyme (pmol his-leu/mg protein/min)	35 ± 38		744±50
Renin (pmol ANG I/mg protein/h)	12_± 17		67±10
Cathepsin D-like acid protease (μg BSA/mg protein/h	1 596±397		726±44

$p<0.01$ $p<0.005$

almost complete degeneration of nerve cells within the corpus striatum of the lesioned rats analyzed

Ibotenic acid produces a selective neuronal degeneration within the corpus striatum without damaging glial elements and extrinsic nerve terminals and axons of passage (Schwarcz et al 1979) The present findings therefore give evidence that the angiotensin-I converting enzyme has a neuronal localization within the corpus striatum These findings are in agreement with the depletion of this enzyme found in the corpus striatum of patients with Huntington s chorea (Arregui et al 1977) The discovery that renin and cathepsin D-like acid protease were increased about 50% following neuronal degeneration of the striatum warrants further methodological and functional investigations It indicates that both enzymes are stored in another cellular compartment than angiotensin I converting enzyme One possibility is that both enzymes are located within glial elements of the corpus striatum since these elements may proliferate following ibotenic acid induced degeneration of the nerve cells Another possibility is that renin is located within the extrinsic nerve terminals of the corpus striatum sin e these terminals remain intact following ibotenic acid induced neuronal degeneration The increase in renin enzyme activity could be looked upon as a compensatory increase due to reduced amounts of angiotensin II present within the corpus striatum in view of the reduction of angiotensin-I converting enzyme activity Renin enzym activity has in fact been demonstrated within synaptosomes (see Ganten et al 1978) This problem concerning the localization of renin will be solved by the use of a proper immunohistochemical analysis of the localization of renin like immunoreactivity It should also be noticed that 50% of the angiotensin-I converting enzyme remains within the corpus striatum which completely lacks nerve cells Thus angiotensin-I converting enzyme is located partly within striatal nerve cells and partly within extrinsic striatal nerve terminals and/or glial elements

In conclusion 50% of the angiotensin-I converting enzyme is located within striatal nerve cells which probably do not contain appreciable amounts of renin indicating that at least in some nerve cells renin and angiotensin I converting enzyme may be differentially stored

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xperimental evidence for neurotensin r a metabolite being a hormone

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eurotensin, a tridecapeptide, is stored in specific ndular cells (N-cells) in the mucosa of the ileum d jejunum and, to a lesser extent, in the colon and odenum (Carraway & Leeman 1976 Helmstaedter et al 1977). Folkers et al (1976) suggested that (Gln⁴)-neurotensin rather than neurotensin may be e naturally occurring peptide In dogs higher concentrations of neurotensin-like immunoreactivity (NTLI) are found in blood draining from the ileum an in arterial blood (Mashford et al 1978) indicating that neurotensin is released into the blood om the ileum. I man food and particularly fat erts a major influence on plasma NTLI Ingestion f fat (55 ml Intralipid 20%) increases the plasma NTLI concentration from 52±7 pM to 109±77 pM n=6) hereas isocaloric amounts of amino acids nd glucose have negligible effects (Rosell & Rökaeus 1979)

In small doses administered i.v to dogs eurotensin or (Gln)-neurotensin inhibits gastric motility (Andersson et al 1977) and suppresses gastric acid secretion (Andersson et al. 1976) It also creases blood flow in the gastrointestinal region nd decreases it in subcutaneous adipose tissue Rosell et al 1976) In man i.v infusion of (Gln)-neurotensin or (Gln⁴)-neurotensin inhibits gastric luces the lower esophageal sphincter (LES) pressure from 14 mmHg to 5 mmHg with return to preinfusion pressure within 15 min after cessation of 5 min infusion period The decrease occurs at plasma NTLI concentrations below those obtained in man after a meal or ingestion of fat (Rosell et al 1980)

The experimental data show that neurotensin or a metabolite, satisfies rigid physiological criteria that should be met before a substance is classified as a hormone with endocrine functions Thus the release of NTLI into the blood is produced by a physiologically meaningful event (ingestion of food especially fat) and gastrointestinal actions following infusion of (Gln⁴)-neurotensin are produced at lower plasma NTLI concentrations than those obtained following ingestion of food Neurotensin or a metabolite may function as an endocrine hormone involved in the postprandial regulation of the gastric acid secretion and the motility of the gastrointestinal tract Thus neurotensin may function as an enterogastrone. In addition neurotensin may regulate the postprandial distribution of fat by adjusting the adipose tissue blood flow

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[Gln⁴]-Neurotensin and gastrointestinal motility in man

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... in the upper digestive tract decreases gastric ... motor activity (Ewald & Boas 1886). The inhibition ... been suggested to be caused by a hormone from ... intestinal mucosa which was named enterogastrone (Kosaka & Lim 1930). Several hormones including gastrin, secretin, cholecystokinin and ... are released after food intake and have ... ascribed to have a role as enterogastrone. ... ever none of them has been shown to be the ... responsible hormone. Neurotensin has pronounced ... effects on motility in the gastrointestinal tract of the ... (Andersson et al 1977) and in man the plasma NTLI increase following ingestion of fat (Rosell & Rökaeus 1979). Neurotensin is therefore an enterogastrone candidate

In order to see if intravenous infusions of (Gln⁴)-neurotensin affect gastrointestinal motor activity in ... the pressures in antrum, proximal and distal duodenum, respectively were measured in six healthy male volunteers. The pressures were measured with three water-filled constantly perfused catheters (0 ... ml min⁻¹) each having a lateral orifice 10 cm apart. (Gln⁴)-neurotensin was infused intravenously (6 pmol kg⁻¹ min⁻¹) during 20 min

During infusion of (Gln⁴)-neurotensin the peristaltic activity (pressure waves above 5 mmHg in antrum progressing to one or two of the recording orifices in the duodenum) was completely abolished ($P<0.001$) from a resting value of 3 4±0 6 contractions ×5 min⁻¹. After cessation of the infusion activity returned to preinfusion levels. Moreover the number of antral contractions above 5 mmHg but without any progression to duodenum was reduced but not totally abolished. The inhibition occurred at plasma NTLI concentrations below those obtained in man after a meal (Mashford et al 1978).

In the fasting state regular motor activity consisting of bursts of contractions migrating caudally and recurring cyclically may be recorded in the small intestine of several species including man (Vantrappen et al 1977). After ingestion of food and especially after ingestion of fat these motor complexes are abolished and the motor activity changes into the "fed" type

In 6 healthy male volunteers the pressures were measured in the proximal duodenum, at the flexura duodenojejunalis and in the jejunum 25 cm distal to the angle of Treitz. Three water-filled constantly perfused catheters (0 08 ml min⁻¹) were used each having a lateral orifice 25 cm apart. (Gln⁴)-neurotensin was infused i v (6 pmol kg⁻¹ min⁻¹) during 200 min

The intervals between the interdigestive migrating motor complexes were significantly ($P<0.001$) prolonged from 111±14 min before infusion to 226±8 min during the infusion period. The motility pattern changed into the fed type during the infusion period. The inhibition of the interdigestive migrating motor complexes also occurred at plasma NTLI levels below those obtained after a meal

(Gln⁴)-neurotensin inhibits the peristaltic activity of the stomach and duodenum and the interdigestive migrating motor complexes in the small intestine in man. The present data support the hypothesis that in man plasma neurotensin or a neurotensin metabolite is an endocrine hormone involved in the postprandial regulation of the motor functions of the gastrointestinal tract.

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Release of neurotensin like immunoreactivity (NTLI) from rat intestine following administration of bombesin

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In man fat ingestion causes a pronounced increase in plasma NTLI presumably due to a direct exposure of the gastrointestinal tract to nutrients but possibly also by the action of some releasing hormone (Rosell & Rökaeus 1979) Bombesin a tetradecapeptide isolated from frog skin (Anastasi et al 1971) has been reported to increase plasma neurotensin levels upon administration to man (Lezoche et al 1979) Recently a porcine gastric heptacosapeptide having striking C terminal homology with bombesin has been isolated (McDonald et al 1979)

The purpose of this investigation was to quantitate rat plasma NTLI increases following parenteral administration of bombesin to attempt localization of the site of NTLI release and to investigate the possibility that a bombesin like substance may be implicated in fat induced elevation of plasma NTLI

In conscious rats tail vein injections of bombesin (7 8 to 250 pmol kg) resulted in dose dependent plasma NTLI increases the maximum increase being obtained with 125 pmol kg Injection of 125 pmol kg increased plasma NTLI to 139±6 4 pM (mean ± S E) 78±4 ? 73±5 1 73±3 4 and 55±3 7 pM at 1 3 5 10 and 20 min respectively (n=6) All values were significantly elevated as compared to a saline control value of 38±1 6 pM at 3 min

Anesthetized rats (n=4) were given ?0 min femoral vein infusions of bombesin at doses of 3 1 and 31 ? pmol kg min respectively An initial peak of plasma NTLI at 3 min was followed by a second gradual rise lasting throughout the remainder of the infusion

Bombesin (125 pmol kg ¹) when injected into the aorta at the level of the celiac artery the sup mesenteric artery or just distal to the sup mesenteric artery with the two other vascular branches clamped (n=5) resulted in significant increase plasma NTLI only after injections into the sup mesenteric artery a maximal value of 102±11 p (basal ?7±3 6) was seen at 45 s

In anesthetized rats intragastric fat administration (? 5 ml Intralipid ?0%) increased plasma NTL from a basal value of 22± 1 pM to 6_±16 55±5 9 at ?0 and 60 min respectively (n 5) However no significant increase of plasma NTLI w seen with intragastric fat when a ligature was pla around the duodenum 1–? proximal to the ligame of Treitz (n=3)

The findings suggest that bombesin relea NTLI in a dose related fashion from the intest region perfused from the sup mesenteric artery (t area corresponding to the location of most of t N-cells) However a bombesin-like substance cated in the proximal gastrointestinal tract is unlik ly to be a mediator of Intralipid induced plas NTLI increase in the rat

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(Gln[4])-Neurotensin and gastrointestinal motility in man

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Fat in the upper digestive tract decreases gastric motor activity (Ewald & Boas 1886). The inhibition has been suggested to be caused by a hormone from the intestinal mucosa which was named enterogastrone (Kosaka & Lim 1930). Several hormones including gastrin, secretin, cholecystokinin and glucagon are released after food intake and have been ascribed to have a role as enterogastrone. However none of them has been shown to be the responsible hormone. Neurotensin has pronounced effects on motility in the gastrointestinal tract of the dog (Andersson et al. 1977) and in man the plasma NTLI increase following ingestion of fat (Rosell & Rökaeus 1979). Neurotensin is therefore an enterogastrone candidate.

In order to see if intravenous infusions of (Gln[4])-neurotensin affect gastrointestinal motor activity in man the pressures in antrum, proximal and distal duodenum, respectively, were measured in six healthy male volunteers. The pressures were measured with three water-filled constantly perfused catheters (0 ml·min[−1]), each having a lateral orifice 10 cm apart. (Gln[4])-neurotensin was infused intravenously (6 pmol · kg[−1] · min[−1]) during 20 min.

During infusion of (Gln[4])-neurotensin the peristaltic activity (pressure waves above 5 mmHg in antrum progressing to one or two of the recording orifices in the duodenum) was completely abolished ($P<0.001$) from a resting value of 3.4 ± 0.6 contractions · 5 min[−1]. After cessation of the infusion the activity returned to preinfusion levels. Moreover the number of antral contractions above 5 mmHg but without any progression to duodenum was reduced but not totally abolished. The inhibition occurred at plasma NTLI concentrations below those obtained in man after a meal (Mashford et al. 1978).

In the fasting state regular motor activity consisting of bursts of contractions migrating caudally and recurring cyclically may be recorded in the small intestine of several species including man (Vantrappen et al. 1977). After ingestion of food and especially after ingestion of fat these motor complexes are abolished and the motor activity changes into the "fed" type.

In 6 healthy male volunteers the pressures were measured in the proximal duodenum, at the flexura duodenojejunalis and in the jejunum 25 cm distal to the angle of Treitz. Three water-filled constantly perfused catheters (0.08 ml · min[−1]) were used, each having a lateral orifice 25 cm apart. (Gln[4])-neurotensin was infused i.v. (6 pmol · kg[−1] · min[−1]) during 200 min.

The intervals between the interdigestive migrating motor complexes were significantly ($P<0.001$) prolonged from 111 ± 14 min before infusion to 226 ± 8 min during the infusion period. The motility pattern changed into the fed type during the infusion period. The inhibition of the interdigestive migrating motor complexes also occurred at plasma NTLI levels below those obtained after a meal.

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Inhibition of pentagastrin stimulated gastric acid secretion and rise in the plasma concentration of neurotensin like immunoreactivity (NTLI) by intraduodenal oleic acid in man

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The effects of intraduodenal administration of oleic acid (5 10, 20 and 40 ml) on gastric acid secretion and plasma concentration of neurotensin-like immunoreactivity (NTLI) were studied in 18 healthy subjects. Gastric acid secretion was stimulated by a submaximal i pentagastrin infusion the amount of which was determined on the basis of individual pentagastrin dose-response tests. We also studied 6 duodenal ulcer patients before and after proximal gastric vagotomy Gastric acid secretion and plasma NTLI were followed during a 120 min period after the intraduodenal installation of oleic acid.

5 ml oleic acid administered intraduodenally significantly inhibited gastric acid secretion by 29% and there was a significant rise in plasma NTLI with peak concentration at 30 min. Maximal inhibition of acid secretion occurred after 20 ml oleic acid (43%). Over the 120 min period there was a close temporal relation between the inhibition of gastric acid secretion and the rise in plasma NTLI In addition, the correlation between individual inhibition of gastric acid secretion and the individual integrated plasma NTLI response to oleic acid was statistically significant. These results together with other data (Andersson et al 1976) suggest that neurotensin or a metabolite may account for the suppression of gastric acid secretion caused by oleic acid. This is in accordance with the hypothesis that neurotensin may have an enterogastrone-like function (Rosell & Rökaeus 1979)

In the duodenal ulcer patients 20 ml of oleic acid evoked a 20% inhibition of gastric acid secretion which was significantly lower when compared to that obtained in the healthy group Moreover the peak plasma NTLI concentrations were significantly lower in the duodenal ulcer patients than in the healthy group Proximal gastric vagotomy abolished the inhibition of gastric acid secretion by oleic acid. The total integrated plasma NTLI response was the same before and after proximal gastric vagotomy These findings confirm earlier results that neurotensin needs an intact vagal innervation to supress gastric acid secretion (Andersson et al 1980) Moreover the data indicate that duodenal ulcer patients may have a defective fat inhibitory mechanism.

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hibition of pentagastrin stimulated gastric id secretion and rise in the plasma ncentration of neurotensin like immunoreactivity TLI) by intraduodenal oleic acid in man

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e effects of intraduodenal administration of oleic id (5, 10, 20 and 40 ml) on gastric acid secretion d plasma concentration of neurotensin-like immunoreactivity (NTLI) were studied in 18 healthy bjects. Gastric acid secretion was stimulated by a bmaximal pentagastrin infusion, the amount hich was determined on the basis of individual ntagastrin dose-response tests. We also studied 6 odenal ulcer patients before and after proximal stric vagotomy. Gastric acid secretion and plasma NTLI were followed during a 120 min period ter the intraduodenal instillation of oleic acid.

5 ml oleic acid administered intraduodenally significantly inhibited gastric acid secretion by 29% d there was a significant rise in plasma NTLI th peak concentration at 30 min. Maximal inhibition of acid secretion occurred after 20 ml oleic acid 0%). Over the 120 min period there was a close temporal relation between the inhibition of gastric cid secretion and the rise in plasma NTLI. In ddition, the correlation between individual inhibition of gastric acid secretion and the individual integrated plasma NTLI response to oleic acid was statistically significant. These results together with other data (Andersson et al. 1976) suggest that neurotensin or a metabolite may account for the suppression of gastric acid secretion caused by oleic acid. This is in accordance with the hypothesis that neurotensin may have an enterogastrone-like function (Rosell & Rökaeus 1979).

In the duodenal ulcer patients 20 ml of oleic acid evoked a 20% inhibition of gastric acid secretion which was significantly lower when compared to that obtained in the healthy group. Moreover the peak plasma NTLI concentrations were significantly lower in the duodenal ulcer patients than in the healthy group. Proximal gastric vagotomy abolished the inhibition of gastric acid secretion by oleic acid. The total integrated plasma NTLI response was the same before and after proximal gastric vagotomy. These findings confirm earlier results that neurotensin needs an intact vagal innervation to supress gastric acid secretion (Andersson et al. 1980). Moreover the data indicate that duodenal ulcer patients may have a defective fat inhibitory mechanism.

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Plasma NTLI after administration of fat to obese volunteers and patients operated with jejuno ileal bypass

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The aim of the present study was to compare the plasma levels of neurotensin like immunoreactivity (NTLI) after the oral administration of fat between healthy and obese volunteers and patients operated for obesity with jejuno-ileal bypass where a large proportion of the N-cell containing small intestine is shunted away from the contact with food

Plasma NTLI was determined after the oral administration of 55 ml 20% Intralipid (11 g of fat mainly as triglycerides) to 3 groups of subjects

(*a*) 6 healthy volunteers weighing on the average 62 kg

(*b*) 6 very obese but otherwise healthy volunteers weighing on the average 137 kg

(*c*) 20 patients who to 10 years previously had been operated with jejuno-ileal bypass and thereafter decreased their average weight from 138 to 87 kg

After fat administration to the healthy volunteers the plasma concentration of NTLI increased significantly from 43±5 pM to 85±13 pM at 30 min In the obese volunteers fat caused an increase of NTLI concentration from 27±6 pM to a maximum of 50±10 pM at 90 min At 30 and 60 min after the fat administration the NTLI concentrations were significantly ($P < 0.01$) lower than for non-obese volunteers In the patients with jejuno-ileal bypass the NTLI concentration increased from 34±4 pM to a maximum of 125±19 pM at 180 min which was significantly ($P < 0.01$) higher than in the obese volunteers

These data indicate that plasma NTLI levels are lower in very obese volunteers than in healthy ones Fat induced increases in plasma NTLI were similar although delayed in obese subjects Moreover in years after jejuno-ileal bypass the NTLI response was larger than in non-operated obese subjects The reasons for these differences are not clear The number of N-cells exposed to ingested fat may have been quite different in the obese and normal subjects After jejuno-ileal bypass the number of N-cells may have increased in the functioning intestine which is well known to hypertrophy (Polak et al 1978 Besterman et al 1978) Alternatively the dose response curve may have been displaced to the left with increased sensitivity of the N-cell for fat Another possibility is that some yet unknown substance released from the proximal intestine may cause the increased response in plasma NTLI

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ological profile of leukotrienes C_4 and D_4

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reacting substance of anaphylaxis (SRS–A) long been recognized as a smooth muscle active compound or family of compounds. As presumed mediator of anaphylactic reactions it has been particularly linked to perturbations of lung mechanics (Orange & Austen 1969) It was recently found that SRS–A (Murphy et al. 1979) belongs to a novel group of compounds the leukotrienes (Borgeat & Samuelsson 1979). These are formed from arachidonic acid by pathway initially involving formation of 5-hydroperoxyderivative (Fig 1). The chemical structures of SRS–A from various sources have recently been determined and the metabolic pathways involved in their formation and further transformation have been elucidated as indicated in Fig 1 (Samuelsson et al 1980). In this communication we wish to report on some biological effects of leukotrienes C and D (LTC_4, LTD_4), with special reference to previous information on biological activities of crude SRS–A. These results were presented at the 1980 Prostaglandin Winter Conference in Snowbird, Utah USA.

The following test systems were used, helical strips of guinea pig bronchi and guinea pig trachea, strips from guinea pig lung parenchyma, ileum (longitudinal muscle layer and/or longitudinal segment) and uterine horn (virgin animals, and adults castrated 4 weeks before the experiment), tracheal insufflation pressure and dermal vascular permeability in guinea pigs (all systems sensitive to SRS–A), and the relatively SRS–A insensitive rabbit duodenal and bronchial strips, and strips from rat uterine horns. Strip tests were performed at 37°C in 5 ml baths containing Tyrode or de Jalon (uterine horn) solutions gassed with 5% CO_2 in O_2. Contractions were recorded isometrically (Grass FT 03) or isotonically (intestinal and uterine strips, Harvard 356, load 0 25–0 5 g). Studies on insufflation pressure and vascular permeability were done according to Strandberg & Hedqvist (1975) and Orange & Austen (1968) respectively.

LTC and LTD (0 1 1 0 nM) caused concentration-dependent contractions of guinea pig ileum, but they did not contract rabbit duodenal strips in concentrations up to 50 nM. The guinea pig ileum is interesting from the point of view that it is a classical SRS–A assay preparation widely used to define the biological activity of one unit SRS–A in relation to that of histamine. In the present study histamine was on a molar basis 200 times less active than LTC suggesting that one unit of SRS–A corresponds to approximately 0.2 pmol LTC.

Likewise LTC dose-dependently contracted uterine strips from virgin (0 1–1 0 nM) and adult ovariectomized (1 0–100 nM) guinea pigs being approximately 300 times more potent than histamine in the two preparations. Uterine horns from rats brought into artificial estrus proved insensitive to LTC (100 nM) and histamine (10 μM) but they contracted vigorously to 100 nM PGE_2.

LTC and LTD were remarkably potent and largely equiactive in causing increased vascular permeability in guinea pig skin. Thus, 0 1 pmol (0.1 ml) injected intradermally was sufficient to produce (in 30 min) blue spots (due to leakage of circulating Evans blue) of significantly greater intensity and size than those caused by NaCl (solvent) or histamine (0 1 nmol).

Pulmonary effects of leukotrienes are of particular interest, considering that SRS–A may have a mediator role in anaphylactic reactions. Intravenous injection (0.2 ml into jugular vein) of LTC and histamine caused dose-related and parallel increases in insufflation pressure in anesthetized and artificially ventilated guinea pigs (Fig.) LTD was virtually equipotent with LTC_4, the $ED_{100\%}$ (the dose that causes 100% increase in insufflation pressure) being 89 and 120 pmol respectively and they were more than 100 times more potent than histamine ($ED_{100\%}$ = 14 nmol). The leukotrienes were at least as potent when they were given as aerosols indicating that the increase in insufflation pressure was due to bronchoconstriction. Notably the results are in excellent harmony with previous information on effects of SRS–A in this test system (Strandberg & Hedqvist 1975).

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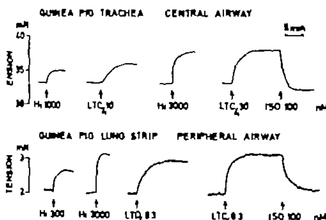


Fig. 3. Contraction responses to histamine (H.), LTC_4, and LTD in strips from guinea pig trachea and lung parenchyma. Reversal of LTC-induced contraction by isoproterenol (ISO).

7 vanished only after repeated washing or administration of the SRS–A antagonist FPL 55712 (10 μM).

The data presented in this communication show that the leukotrienes C and D are equally and remarkably potent in a number of organs. In particular those involved in anaphylactic reactions and that they have a biological profile, which strikingly correlates with that of impure preparations of SRS-A (cf. Orange & Austen 1968). This strongly supports the concept that SRS–A consists of leukotrienes LTC and LTD_4, formed from arachidonic acid by the lipoxygenase pathway given in Fig. 1. LTC_4 is easily converted into LTD by the enzyme γ-glutamyl-transpeptidase and the relative proportions of these two leukotrienes in in vitro preparations therefore depend on incubation conditions (cf. Bach et al. 1979). Whether LTC or LTD is the principal mediator in anaphylactic reactions in vivo remains to be established. However there does not seem to be any significant difference in their effects on the most sensitive respiratory muscle preparations, i.e. the peripheral airways.

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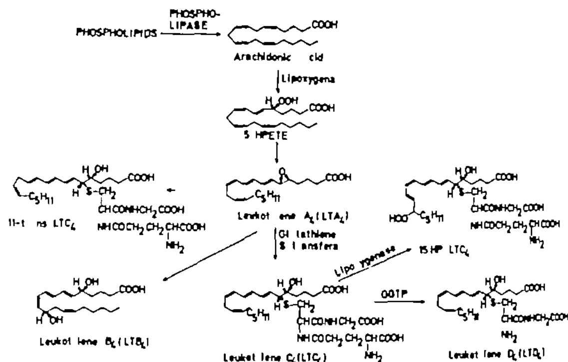


Fig 1 Schematic representation of transformations of arachidonic acid into leukotrienes 5-hydroperoxy-6,8,11,14-eicosatetraenoic acid (5-HPETE) 5(6)-oxido-7 9 11 14-eicosatetraenoic acid (LTA) 5 1 -dihydroxy-6,8,10,14-eicosatetraenoic acid (LTB) 5-hydroxy 6S-glutamylcysteinylglycyl-7 9 11 14-eicosatetraenoic acid (LTC), 5-hydroxy-6-cysteinylglycyl-7 9 11 14-eicosatetraenoic acid (LTD) 5-hydroxy 15-hydroperoxy 6S-glutamyl-cysteinylglycyl-7 9 11 13-eicosatetraenoic acid (15HP LTC)

The pulmonary effects of LTC and LTD were further characterized in isolated strips from guinea pig human and rabbit lung. As illustrated in Fig 3 LTC and LTD were largely equipotent in con-

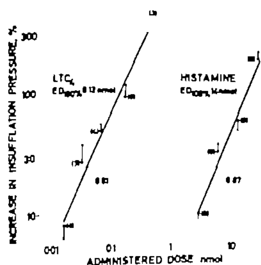


Fig 2 Increase in insufflation pressure induced by intravenous injections of LTC and histamine in guinea pigs Shown are mean values ± S E. (n)=number of observations from 1 expts Regression lines according to the method of least squares

tracting guinea pig parenchymal or tracheal strip The observation that lower concentrations of leukotrienes were required for significant contraction of parenchymal than tracheal strips, and that the histamine to leukotriene molar ratio for equivalent contraction amplitudes was more than 100 1 in trachel strips and more than 1 000 1 in parenchymal strips seems to indicate that leukotrienes are particularly active on peripheral airways as previously suggested also for SRS–A (Drazen et al 1979)

Rabbit bronchial strips did not respond to leukotrienes in concentrations up to 100 nM whereas human bronchi again proved remarkably sensitive to LTC and LTD Thus 0 1 nM of either leukotriene was sufficient for a significant response and 100% increase in tension was obtained with 6 nM LTC as compared with 10 μM histamine. This would seem to make the leukotrienes outstanding as bronchoconstrictors in man

In all types of smooth muscle strips contractions induced by LTC or LTD invariably developed after a longer latency and at a considerably slower rate than those induced by histamine Furthermore they were consistently longlasting and unaffected by atropine (0 3 μM) o ramine (1 μM), and

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ree fatty acids in the brain in bicuculline-induced tatus epilepticus

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though it is well known that prolonged or re ated epileptic seizures lead to neuronal cell dam- e in the brain the mechanisms of damage have mained undefined. Experiments on bicuculline luced status epilepticus in the baboon and the rat ve shown that neuronal lesions develop in some lectively vulnerable areas (predominantly neocor s and hippocampus) in spite of artificial ventila- n and a seemingly adequate oxygen supply (Mel- um et al 1973 Blennow et al. 1978) and in spite minimal perturbation of cerebral energy state hapman et al 1977). These results suggest that e cell damage is at least partly unrelated to energy ilure (see Blennow et al 1978). During recent ars, it has become increasingly evident that ll damage may be elicited by oxidative break wn of arachidonic acid via the cyclo-oxygenase Kirtcloutis & Garbus 1975 Flower 1979) and oxygenase pathways (Borgeat & Samuelsson 79) The question therefore arises whether achidonic acid accumulates during status epi- pticus. Previously elevated concentrations of lyenoic free fatty acids (FFA) have been meas- ed in seizures provoked by electro-shock and ntylenetetrazol (see Bazán 1976 Marion & Wolfe 78) However since the animals used were eathing spontaneously the possibility remains at respiratory distress contributed to the develop- ent of gross energy failure. In order to clarify hether or not FFA accumulate in the brain during izures in the absence of such energy failure we easured FFA concentrations in the cerebral cor ex of rats in bicuculline-induced status epilepticus.

Methods Male Wistar rats (250–400 g) were allowed free ccess to rat pellets and tap water Anesthesia was induced ith halothane (2 3%), maintained during operative pro- dures on 1% halothane and 70% N_2O in oxygen, and then continued on 70% N_2O and 30% O_2 with artificial ventilation Catheters were placed in femoral artery and vein, EEG electrodes were inserted into the skull bone and provision were made for freezing the tissue in situ. Following adjustment of body temperature (37°C), arterial P_{O_2} (>100 mmHg) and P_{CO_2} (35–40 mmHg), seizures were induced by i. injection of bicuculline (1.2 mg kg) Cortical tissue was frozen for metabolite analyses after 1 30, and 60 min of continuous seizure activity

Frontoparietal cortical tissue from one hemisphere was extracted with HCl-methanol and subsequently with perchloric acid for enzymatic fluorometric analyses of adenine nucleotides (ATP ADP and AMP for references to analytical techniques, see Chapman et al 1977). A corresponding area from the other hemisphere was extracted with chloroform-methanol for the subsequent separation and analysis of FFA with thin layer and gas-liquid chromatography (for analytical techniques, see Rehncrona et al 1980).

Results and Discussio The cortical FFA concentration in control animals was 0 16 ±0 005 μmol g (means ± S E. $n=6$). After 1 30 and 60 min of seizure activity the concentrations rose to 0.365±0.04 ($n=5$) 0 41 ±0 041 ($n=6$) and 0.229±0 15 μmol g respectively As Fig. 1 shows the increase in arachidonic acid (20 4) and stearic acid (18 0) was even more pronounced while there were no significant changes in palmitic (16 0) or oleic (18 1) acids. The concentration of the polyenoic docosahexaenoic acid (22 6) rose 4- to 6-fold (data not shown)

At 1 min there was a moderate but clear pertur bation of cerebral energy state with changes in ATP ADP and AMP concentrations (cf Chapman et al 1977) However at 30 and 60 min cerebral energy state was minimally perturbed For ex ample at 30 min the ATP ADP and AMP concentrations were 2.62±0 02, 0.279±0.011 and 0 041±0 003 μmol g respectively while control concentrations were 2.77±0 01 0.278±0 009 and

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Estimation of capillary permeability of inulin sucrose and mannitol in rat brain cortex

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AMTORP O Estimation of capillary permeability of inulin sucrose and mannitol in rat brain cortex Acta Physiol Scand 1980 110 337 342. Received 8 May 1979 ISSN 0001 6772. Institute of Medical Physiology A, University of Copenhagen, Denmark.

The present study analyzed the brain uptake of the differently sized hydrophilic molecules ^{14}C-inulin, ^{14}C-sucrose and ^{14}C-mannitol Constant tracer concentrations were maintained in blood plasma after renal ligation. Accumulation of the indicators was measured in brain cortex during the approach to steady state At the conclusion of infusion periods of 5 10, 15 30 and 60 min, samples of cerebral cortex were analyzed for radioactivity Fractions attributable to blood in the tissue were subtracted and time-dependent apparent distribution volumes of the indicators in the tissue were estimated. The brain level did not rise to more than 1 % of that in the plasma for inulin and sucrose and to 6–7% for mannitol. The explanation for this could be combination of restricted penetration from blood into brain and sink effect of cerebrospinal fluid (csf). T determine the removal of the indicators into csf ventriculo-cisternal perfusion was performed during the period of tracer uptake in the tissue; the rate of passage of the indicators into perfusion fluid was found to be negligible in comparison to the rates of uptak in the tissue. As diffusion in the e tracellular space could limit uptake rates from blood to brain, estimates of diffusion limited half-times were also made Calculations showed that the major hindrance to indicator uptake in the tissue is located in the wall of the brain capillaries, thus the uptake data permit calculation of brain capillary permeability The half-times for the distribution of the indicators in their equilibrated tissue distribution spaces were used to estimate brain capillary permeability By comparison with the aqueous diffusion coefficients of the indicators it is concluded that the substances in the molecular weight range of 182 to 5 900 daltons are subjected to restricted diffusion during the passage of the blood-brain barrier and that in addition non-discriminative transendothelial pathways are available.

Key words ^{14}C Labelled inulin, sucrose and mannitol, blood-brain barrier permeability

Estimates of brain capillary permeability coefficients have been obtained by the aid of the indicator diffusion method (Crone 1965), based on solute diffusion, i e, loss of material from blood as it flows through the brain in a single passage This method is inapplicable to solutes with low rate of transcapillary transport due to unavoidable inaccuracies in determining very low extractions (Lassen et al 1971) The only method which can be used involves tissue sampling at various times after a continuous vascular infusion (Patlak & Fenstermacher 1975).

In the present study the tissue uptake method (Johnson & Wilson 1966) based on measurements of uptake of solutes with time under conditions with constant concentration of indicator substance in blood, was used. Using radioactive indicators I have followed the permeation of cortical tissue by inulin sucrose and mannitol which all have small capillary permeability coefficients and which are essentially confined to the extracellular compartment.

The aim of the experiments was to obtain information on processes involved in the passage of the substances across the blood-brain barrier

METHODS

Male Sprague-Dawley rats weighing 300–350 g were used

Details on animal preparation and perfusion technique have been given previously (Amtorp 1979) In short, they were as follows: Anesthesia was introduced by i p pentobarbital (50 mg/kg) and maintained by injections as required Both kidneys were exposed by retroperitoneal approach and the renal pedicles ligated. Tracheostomy and intubation was made Catheters were introduced into

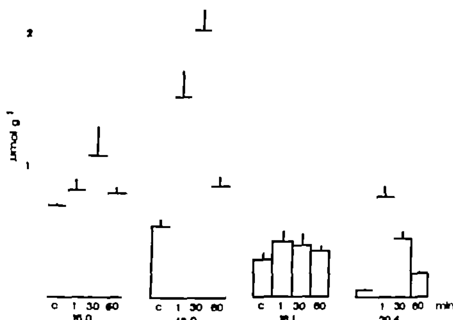


Fig 1 Changes in the cerebral cortex concentrations of some free fatty acids during bicuculline-induced status epilepticus of 1 30 and 60 min duration The values are means ± S E in µmol g of wet weight for groups of 6 animals C = control $P<0.01$ $P<0.001$

0 044±0 003 µmol respectively A comparison with Fig. 1 shows that in spite of this near normal energy state the arachidonic acid concentration had increased more than 6-fold

The results allow two main conclusions First a marked accumulation of arachidonic acid occurs and is maintained during seizures even though gross energy failure is absent Second since this accumulation occurs in a tissue whose oxygen supply is upheld the conditions for an arachidonic acid cascade are at hand Conceivably oxidative metabolism of the arachidonic acid in the cycle-oxygenase and the lipoxygenase pathways contributes to the cell damage occurring

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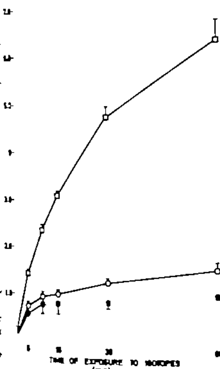


Fig. 1. The time course of uptake of inulin ●—● sucrose ○—○ and mannitol □—□ by rat cerebral cortex expressed as apparent spaces. Each point represents the mean values of 5 to 7 rats. The bars indicate ±1 S.E.

expts. in which ^{14}C-mannitol was infused for 60 min, 30 plasma samples were available. Taking the mean concentration in each expt. as 1.0 the regression of the concentration upon time was 1.022+0.0002 t (SE=0.018) for inulin 1.001+0.0013 t (S.E.=0.022) for sucrose and 1.027+0.0010 t (S.E. 0.013) for mannitol

The uptake by brain cortex of ^{14}C-inulin ^{14}C-sucrose and ^{14}C-mannitol expressed as apparent spaces versus time of exposure to the indicators is given in Fig. 1

In Table 1 are presented the rate constants for the penetration from blood to brain cortex of ^{14}C-inulin, ^{14}C-sucrose and ^{14}C-mannitol obtained as described. The relatively low correlation coefficients of the regression lines calculated for ^{14}C-inulin and ^{14}C-sucrose are due to the low slopes of the regression lines. Also included are the values of equilibrium extracellular distribution volumes in cortical tissue (V_{ec}). The capillary permeability-surface area products (PS) as the best working measure of barrier permeability were calculated from the rate constants and equilibrium distribution volumes

DISCUSSION

In this study an attempt was made to determine brain capillary permeability to molecules which have small permeability coefficients. The method used and its application to various tissues has been discussed in detail (Johnson & Wilson 1966 Johnson 1970 Wittmers et al 1976)

When considering the kinetics of uptake by cortical tissue several problems may complicate the analysis of the data. The choice of inulin sucrose and mannitol as test substances which are inert of nature avoids problems concerned with influence of chemical processes upon the kinetics of uptake by the tissue The contents of the indicators in cortical tissue was obtained by subtraction of residual amounts of inulin sucrose and mannitol in the vascular compartment, which allowed calculation of the tissue volumes of distribution at the time the sample was taken. Correction was obtained in the individual experiments by use of ^{113m}Indium bound to plasma transferrin as a plasma tracer By this procedure the vascular plasma compartment was found to be 0 44 µl 100 mg with a variation of 6%

A substance passing out of the blood capillaries first enters the extracellular space and from there it will pass into the cells if the cell membranes are permeable to the substance. The particular substances being studied here are essentially confined to the extracellular compartment and the uptake by brain cortex was considered to occur exclusively in the extracellular fluid in the time periods studied The equilibrium extracellular volumes of distribution in rat brain cortex have been measured recently (Amtorp 1979). The distribution spaces of ^{14}C-inulin, ^{14}C-sucrose and ^{3}H mannitol were determined following tracer equilibration times of 5 h with combined intravenous infusion and ventriculo-cisternal perfusion, the aim being to preclude sink action of csf during equilibration (Oldendorf & Davson 1967) The variability (S D 100/mean) of the equilibrium volumes of distribution was of the order of 7% for ^{14}C-inulin and of the order of 13% for ^{14}C sucrose and ^{3}H-mannitol It is evident that there was some scatter in these data, which affects the accuracy of the estimates of capillary permeability

Opportunities exist for diffusional exchange of solutes between brain extracellular fluid and csf

a femoral artery and vein The arterial blood pressure was continuously recorded Body temperature was maintained at 37 8–38.2°C The animals were immobilized with gallamonium iodide (20 mg/kg) Artificial ventilation with *ambient air was maintained with a small animal respirator* and pH P and P were measured (BMS 3 Mk Blood Micro System Radiometer Copenhagen)

With the aid of a stereotactic device (Kopf Instruments) two inflow cannulae were placed one in each lateral ventricle The ventricular system of the brain was perfused with artificial csf The rate of infusion delivered from a slow infusion pump (Braun-Melsungen) was 15 1 μl/min with one half diverted to each lateral ventricle The atlanto-occipital membrane was exposed and a cisternal cannula was inserted into the cisterna magna. Outflow fluid was collected via polyethylene tubing into glass vials placed close to the experimental animals in order to minimize the *dead space i e the perfusion volume between cisterna* magna and the test tubes (estimated to less than 700 μl) Collection of fluid was carried out under a negative pressure of 10 cm H O the aim being to keep the pressure in csf in the subarachnoid space below that in the venous sinuses

Inulin sucrose and mannitol were the test molecules utilized in the experiments. C labelled inulin sucrose and mannitol were obtained from New England Nuclear Corp *Boston Mass. USA* The purity of isotopical inulin sucrose and mannitol was verified by gel filtration using a G-25 Sephadex column (Pharmacia) The isotopic test molecules were prepared in isotonic NaCl

The volume of residual blood in the brain was estimated using Indium bound to plasma transferrin as a plasma tracer (Hossain et al 1969) Freshly generated Indium obtained from a sterile ion exchange generator was mixed with arterial blood and injected i v 5 min before the end of the experiments.

To determine the time course of permeation of the indicator molecules from plasma into brain a solution of the test substance was infused into a femoral vein In preliminary experiments on rats with ligated renal vessels, plasma clearance of C-labelled inulin sucrose or mannitol was determined from multiple small plasma samples obtained at variable time interval between 1 min and 1 h after i v injection. Using the plasma clearance curves an empirical infusion schedule was designed to give approximately constant plasma concentration

Sampling of tissue and fluids The effluent csf was collected continuously during periods of 5 min and effluent volumes were determined by weighing At a set time samples of arterial blood plasma were assayed for radioactivity and the animals were killed by decapitation and exsanguinated to minimize the vascular blood content of the brain The brain was rapidly removed and divided into separate hemispheres *The layer of cerebral cortex* was separated by peeling off subcortical tissues and careful removal of the meninges.

Analyses A Packard Tri-Carb liquid scintillation spectrometer system model 2425 was used to count carbon-14 labelled compounds Indium (U_1=99 5 min) was allowed to decay before beta counting commenced 25 μl plasma and 50 μl perfusion fluid were placed in scintillation vials. Pieces of brain were weighed in scintillation vials. Beta counting was performed after tissue solubilization 10 ml scintillator solution (Instagel Packard) were added to all the vial which were kept in darkness at 4°C for 1 h to eliminate chemiluminescence Quench correction was calculated using external standard channel ratio method Gamma emission from Indium was counted in a well crystal counter (Packard Autogamma, model 5...) and correction for decay during the counting period was made

Calculations After appropriate background and efficiency corrections brain tissue activity of the test molecule (cpm/g wet weight) was obtained and the fraction attributable to blood remaining in the brain was subtracted The apparent extravascular distribution volume in the tissue (V) was then calculated as

$$V = \frac{\text{activity per 100 mg tissue}}{\text{activity per } \mu l \text{ plasma water}} (\mu l \ (100 \text{ mg})^{-1})$$

The values of equilibrium extracellular distribution volumes (V_{eq}) of the indicators in brain cortex were obtained *from a recent study* (Amtorp 1979) The rate constants for penetration of the solutes into brain were obtained by calculating the regression lines for the ratio log (1 V/V_{eq}) vs time The permeability surface area product (PS) with dimensions of length³ time ma s equals the product of the rate constant (k) and the equilibrium volume of distribution (V_{eq}) (Johnson & Wilson 1966, Wittmers et al 1976)

$$PS = k \cdot V_{eq} (\text{ml } (100 \text{ g}) \ \text{min} \ ) \quad (1)$$

The assumptions underlying this equation are (1) Homogeneity of exchange vessels with respect to permeability ( ) The resistance to diffusional distribution of indicator material within the tissue distribution space is much lower than that offered by the endothelium of the exchange vessels, i e the tissue distribution space can be considered well-mixed (3) Indicator material cannot leave the tissue space by any other routes than the vascular endothelium through which it has entered the tissue The last assumption implies that passage of indicator material from the tissue to csf must be negligible

RESULTS

Tidal volume and rate of respiration was adjusted using a constant volume respirator *in order* to keep arterial carbon dioxide pressure (P) between 30 and 40 torr Arterial blood pressure averaged 98 mmHg (range 75–135 mmHg)

The concentration of C inulin C sucrose and C mannitol attained in blood plasma of nephrectomized rats was analysed in preliminary experiments In 5 expts in which C inulin was infused for 60 min 53 samples of plasma were available in 5 expts in which C sucrose was infused for 60 min 54 plasma samples were available and in 5

Table 3 *Physical parameters of te t molecule*

The diffusion coefficient in water at 38°C was calculated from D_w by the relation

D_w D (311/293) (η / η_{38})

where η the viscosity of water at indicated temperature. Stokes Einstein hydrodynamic radius = RT/6$\pi$$\eta$ DN. R the gas constant, T absolute temperature, η viscosity of water, D = free diffusion coefficient of solute in water and N Avogadro's number

Compound	D_w cm²·sec⁻¹ 10⁶	Hydrodynamic molecular radius cm 10⁸
Inulin	2.5	13 1
Sucrose	7.[illegible]	4.6
Mannitol	8 8	3 7

The conclusion that the rate limiting step of the passage of the substances from blood into cortical tissue is transfer across the capillary membrane

The calculation of brain capillary permeability from the time course of uptake of indicators into brain cortex is based upon the basic assumption that the rate of passage of the indicators is at any time proportional to their concentration differences across the blood-brain barrier i e that the passage occurs by diffusion and/or bulk transport symmetrically with respect to the barrier By use of differently sized indicator molecules present data may contribute to our understanding of mechanisms of transcapillary movement such as non-restricted diffusion or restricted diffusion Non-restricted diffusion through water-filled channels implies that indicator molecules with different molecular size diffuse across the capillary membrane at rates proportional to their respective aqueous diffusion coefficients. The concept of restricted diffusion implies a deviation of the ratio between the permeability coefficients from the ratio between the aqueous diffusion coefficients Some physical parameters of the indicator molecules are presented in Table 3 The ratio D (mannitol)/D (sucrose) = 1.22 and the ratio D (mannitol)/D (inulin) = 3 52 The corresponding ratio between the calculated PS-values were 4.90 and 8 00) respectively These PS ratios are not commensurate with non-restricted diffusion in water filled channels through or between capillary endothelial cells Permeability decreased disproportionally with increasing molecular size the quoted ratios of permeability coefficient being larger than the ratios of corresponding aqueous diffusion coefficients suggesting that entry of the solutes into brain cortex is governed by a sieving process However the ratio of permeability coefficients between ¹⁴C sucrose and ¹⁴C-inulin was lower than the ratio between the aqueous diffusion coefficients which is incompatible with restricted diffusion through aqueous pores as the only route of passage of the substances across the blood-brain barrier This is so whether the barrier has pores of uniform or distributed size A more complex model such as one with pores plus bulk transport in vesicles is required to explain the data (cf Garlick & Renkin 1970 Carter et al. 1974)

The most likely interpretation of the experimental data is that there are two pathways for the passage of the substances a set of pores which allows passage by restricted diffusion and a non-discriminative process The former would account for the major part of the passage of mannitol while the latter would be responsible for a considerable part of the passage of sucrose and, in particular of inulin The non-discriminative process probably consists of a set of pinocytotic vesicles which open to the luminal surface of the capillary membrane during formation and include indiscriminately in the vesicular volume anything that is originally in solution in the plasma Thereafter the vesicles exchange their contents by transit across the endothelial cells. The motion of free vesicles within the cytoplasma of the endothelial cells is assumed to be Brownian (Karnovsky & Shea 1970) The contribution of vesicular transport to solute permeability equals the rate of turn-over of the vesicles (Renkin 1964 Palade & Burns 1968 Tomlin 1969 Shea et al 1969)

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Table 1 *Capillary permeability surface area products (PS)*

In the first column are given the compounds to which the values in the same row of the subsequent columns are related. The second column gives rate constants for the uptake of the compounds The third column gives correlation coefficients for linear regression lines from which rate constants for uptake were determined. The fourth column gives the equilibrium volumes of distribution The fifth column gives the calculated permeability surface area products

Compound	k (min)	r	V_m (ml 100 g)	PS (ml (100 g) min)
¹⁴C Inulin	0 0011	0 83	13 3	0 015
¹⁴C Sucrose	0 001	0 84	16 6	0 020
¹⁴C Mannitol	0 006_	0 97	19.5	0 121

which may contribute to underestimate indicator uptake by brain cortex and should be taken into account (Welch 1969) The total volume of csf is relatively small about 10% of brain weight (Bradbury 1979) It is in contact with the central nervous tissue over considerable areas of the ependymal and pia glial membranes In the rat pia and arachnoid are in opposition in some places but in others separate to enclose cisterns of csf Over the cerebral cortex these cisterns are absent and subarachnoid csf should not be regarded as bathing the whole of the external surface of the cortex (Bradbury 1979) The flow of csf in cerebral ventricles and subarachnoid space is governed by small hydrostatic pressure gradients generated by its production in the choroid plexus The drainage of csf is largely via the arachnoid villi (Davson et al 1970) During ventriculo-cisternal perfusion with pressure about −10 cm H O the arachnoid villi collapse (Pappenheimer et al 1962) In the present expts csf in juxtaposition to the cerebral cortex should therefore be rather stagnant and only able to leave by way of the outflow cannula in the cisterna magna Analysis of the indicator contents in the outflow fluid allowed estimation of the over all losses by way of csf Accumulation of indicator material in outflow perfusion fluid during ventriculo-cisternal perfusion was compared with accumulation in the tissue during periods of tracer uptake It was found that the removal by the perfusion fluid amounted to only 5–7% of indicator substance accumulated in brain cortex Since a major part of indicator material entering the perfusion fluid by passage into the ventricular cavities must arise from blood passing vessels in the vicinity of the ventricles and possibly the choroid plexus the sink effect of perfusion fluid and subarachnoid csf with respect to the cerebral cortex must be considered negligible

In order to quantitate the indicator uptake data [in] terms of capillary permeability some assumptio[ns] were made about the system (Johnson 1970) Rad[ial] mixing of the indicator material within the cap[il]laries seems fulfilled in this study since capill[ary] diameter of 4 8×10⁻⁴ cm (Wiederhold et al 1976) [is] small and diffusion coefficients are relatively h[igh] (>2×10⁻⁶ cm² sec) Diffusional mixing in the [ex]travascular distribution volume was evaluated [us]ing an equation employed by Schafer & Johnson (197[-]) which yields an expression for diffusion limit[ed] half times

$$t_{\frac{1}{2}} = 0\ 693\ (RV/2\ AD^*)$$

The apparent diffusion coefficients (D*) for the [in]dicator molecules in brain tissue were taken fr[om] Fenstermacher et al (1970) Available data [-] capillary surface area (A=240 cm² g⁻¹) [-] maximum diffusion distance (R=16·10⁻ [-] (Sjostrand 1935) were used Table 2 summari[zes] diffusion limited half times calculated from eq[ua]tion (2) and the half times for tracer uptake fro[m] capillary blood calculated from present experime[n]tal data A comparison of the half-times suppor[ts]

Table 2 *Estimated half times for diffusion equilibration in brain tissue and the half-times f[or] cortical uptakes calculated from present exper[i]mental data*

Compound	Diffusion $t_{\frac{1}{2}}$ (min)	Observed $t_{\frac{1}{2}}$ (min)
¹⁴C Inulin	18	626
¹⁴C Sucrose	11	591
¹⁴C Mannitol	13	11

Table 3. Physical parameters of test molecules

The free diffusion coefficient in water at 38°C was calculated from D_w by the relation

$D_{38} = D_w \cdot (311/293) (\eta_{20}/\eta_{38})$

where η the viscosity of water at indicated temperature. Stokes Einstein hydrodynamic radius = RT/6πη DN the gas constant, T absolute temperature, η viscosity of water, D = free diffusion coefficient of solute in water and N Avogadro number

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The most likely interpretation of the experimental data is that there are two pathways for the passage of the substances a set of pores which allows passage by restricted diffusion and a non-discriminative process. The former would account for the major part of the passage of mannitol while the latter would be responsible for a considerable part of the passage of sucrose and in particular of inulin The non-discriminati e process probably consists of a set of pinocytotic vesicles which open to the luminal surface of the capillary membrane during formation and include indiscriminately in the vesicular volume anything that is originally in solution in the plasma. Thereafter the vesicles exchange their contents by transit across the endothelial cells. The motion of free vesicles within the cytoplasma of the endothelial cells is assumed to be Brownian (Karnovsky & Shea 1970) The contribution of vesicular transport to solute permeability equals the rate of turn-over of the vesicles (Renkin 1964 Palade & Burns 1968, Tomlin 1969 Shea et al 1969)

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Adjustments of hepatic and small intestine blood flow on selective vasoconstrictor fibre stimulation

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The mutual changes in hepatic and small intestinal blood flow on selective nervous stimulation of the periarterial vasoconstriction fibres, were studied in anaesthetized cats. Occlusion of the hepatic artery did not change portal blood flow whereas occlusion of superior mesenteric blood flow caused a significant increase in hepatic arterial flow. Stimulation of the hepatic sympathetic nervous supply caused a phasic blood flow response with a marked transient peak flow reduction of hepatic arterial blood flow. The magnitude of the peak response varied with the frequency of the stimulation. Despite continuous stimulation the first phase went over into a second phase of less pronounced vasoconstriction. This "steady state" blood flow was maintained at about 20% below the control level, irrespective of stimulation frequency. Corresponding in time with the peak vasoconstriction there was a transient increase of portal pressure. Sympathetic nerve stimulation increased portal pressure even on occlusion of the hepatic artery. Stimulation of the mesenteric sympathetic nerves evoked the characteristic transient peak vasoconstrictor response consisting of two phases, a brief intense peak resistance response followed by a second phase of less pronounced but generally well maintained constriction. Simultaneously a slight reduction of portal pressure and hepatic arterial vascular resistance was regularly seen. In contrast to the observations in the hepatic arterial circuit the magnitude of this "steady state" blood flow in the small intestine was dependent on the rate of the stimulation, however. On simultaneous stimulation of the hepatic and mesenteric sympathetic nerves the hemodynamic responses were largely the same as when these nerves were stimulated separately. The portal pressure affecting mean capillary pressure in the intestine differed, however. Small and variable pressure changes were followed by rapid return towards control and during steady state it did not differ from the prestimulatory level.

Many studies on the inherent myogenic and sympathetic nervous control of hepatic and intestinal blood flow have been reported (For review, see Greenway 1963, Folkow & Neil 1971, Greenway & Stark 1971.) Common for these studies are that each vascular bed has been studied separately and don't permit conclusions regarding the intimate interaction between intestinal and hepatic blood flow such as it occurs during simultaneous graded stimulation of the sympathetic nerves to both vascular beds.

The purpose of the present investigation was to study qualitatively and quantitatively the mutual changes of hepatic and small intestinal blood following selective graded electrical stimulation of the periarterial vasoconstrictor fibres, supplying these vascular circuits. It was also considered of interest to study changes in the blood flow and portal vein pressure during vasoconstrictor fibre activation when either or both circuits were temporarily occluded.

METHODS

Operative procedures. Experiments were performed on 20 cats, weighing 4–4.4 kg. The animals, fasted for 24 h, were anaesthetized i.v. with chloralose (50–70 mg/kg) after induction with ether. A tracheal cannula was inserted to maintain a free airway. The abdomen was opened in

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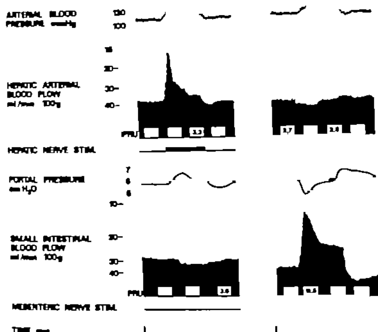


Fig. 2. Cat 3.1 kg. The effects of hepatic (left panel) and mesenteric (right panel) nerve stimulation (8 Hz, 5 ms, 1 V) on systemic arterial blood pressure, hepatic arterial blood flow, portal pressure and small intestinal blood flow. Note the more short-lasting peak response and the more pronounced escape from the vasoconstrictor activation in the hepatic vascular bed. Note also that the portal pressure approaches the control level during sustained stimulation.

blood flow to about 100 ml/min × 100 g tissue. No significant change in portal pressure was observed. When administered in the superior mesenteric artery isoprenaline increased small intestinal blood flow to 200–250 ml/min × 100 g tissue and the portal pressure to about 100 % of the control value, which ranged between 2.9–1 cm H_2O (mean 5.8 ± 0.2). The blood flow levels correspond to those previously reported on cats where enous circuits were used (Folkow, Lundgren & Wallentin 1963, Greenway, Lawson & Mellander 1967), indicating a well maintained myogenic tone in the present series of experiments despite the use of arterial circuits. In the present preparation the portal blood flow equivalent to the superior mesenteric arterial blood flow as calculated to 37 ± 2.1 ml/min × 100 g liver tissue. Except for the pancreas the blood flow in the stomach and the colon when studied by enous or arterial circuits is somewhat lower than in the small intestine and amounts to about 70 ml/min × 100 g tissue (Martinsson 1965, Hultén 1969, Fasth & Hultén 1973, F sth & Martinsson 1973). The resting portal blood flow in the anesthetized cat with the entire splanchnic vascular bed intact can be calculated roughly to about 80 ml/min × 100 g liver tissue. The corresponding reduction of the portal flow in the present study seems to affect the magnitude of the portal pressure but little as this has been reported to range between 5–15 cm H_2O in anesthetized cat with almost intact splanchnic circulation (Greenway et al. 1967).

II. *The eff cts of graded sympathetic nerve stimulation*

A. *Hepati nerves.* The neurogenic constriction of the hepatic resistance vessels exhibited two phases. The threshold value for evoking a response was 0.5 Hz. As is shown in Fig. left panel, there was first a brief but intense reduction of hepatic arterial blood flow (peak response). The magnitude of the initial peak flow reduction showed a linear correlation to the logarithm of the stimulation frequency (= 0.70), 16 Hz causing almost circulatory arrest (Fig. 3, left panel). When calculated as changes in PRU_{100} the peak response at 8 Hz and 16 Hz corresponded to an increase of resistance to flow amounting to 600–1000 %. Despite continuous stimulation this initial flow reduction subsided after 20–30 and

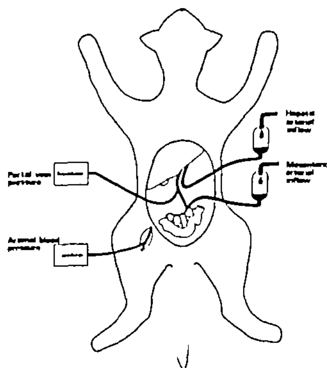


Fig 1 Schematic illustration of the technique used for synchronous studies of small intestinal and hepatic arterial blood flow Note that small intestinal blood flow reflects portal flow

the midline and the greater omentum the spleen the stomach the duodenum the pancreas and colon were extirpated To avoid cholestasis the common duct was cannulated and drained The animals were heparinized (3 mg/kg)

Blood flow and blood pressure recording To measure the hepatic arterial blood flow a wide bore polyethylene tube was inserted into the centrally cut left carotid artery and the blood flow was diverted to a closed perspex optical drop counter filled with silicone oil in turn connected by a short wide bore polyethylene tube to the coeliac artery supplying only the liver and the gallbladder For simultaneous recording of the overall small intestinal blood supply a similar drop recording unit was utilized connecting the left femoral artery with the centrally cut superior mesenteric artery (Fig 1) The drop recorders operated ordinate writers in which the ordinates were inversely proportional to the rate of blood flow The blood flow was recorded on a Grass Polygraph model 7D For continuous recording of portal pressure a polyethylene tubing was inserted via the splenic vein into the portal vein and connected to a Statham pressure transducer (P23AC) operating in ink-writer The right femoral artery was connected to another pressure transducer for recording of systemic arterial blood pressure In control experiments the hepatic arterial perfusion pressure was simultaneously recorded from a T-piece in the tubing connecting the left carotid artery to the coeliac artery close to the point of cannulation of the coeliac artery No significant difference between this pressure and systemic arterial pressure as recorded from the femoral artery was observed and the T-piece was therefore not used routinely because of the space limitation in the region

Nervous stimulation and drug administration The periarterial nerves along the coeliac artery and the superior mesenteric artery were separated and cut immediately where they emerged from the sympathetic ganglion. The peripheral ends of these trunks, containing the postganglionic sympathetic nerve fibres to the liver and small intestine respectively were dissected free and mounted on silver ring electrodes for subsequent selective stimulation Supramaximal square wave impulses (12 V 5 ms) were delivered from a Grass stimulator model S4 The nerves were stimulated at 0 1 16 Hz for 4-10 min Isoprenaline sulphate dissolved in 0 9% saline was administered as graded close infusions into the hepatic and superior mesenteric arteries by means of an automatic infusion pump at rates which did not exceed 0 4 ml/min

Calculation of results Hepatic arterial vascular resistance was calculated as systemic mean arterial pressure (= hepatic arterial mean perfusion pressure) divided by hepatic arterial blood flow (ml/min×100 g liver tissue) Inferior vena cava pressure was in three experiments measured by means of a catheter passed via the right external jugular vein and placed with its tip at the level of the hepatic vein The mean pressure varying between 1-3 cm H_2O remained unchanged during the nerve stimulation and was therefore not taken into account in calculation of the hepatic arterial vascular resistance Superior mesenteric arterial vascular resistance was calculated as the ratio of mean arterial pressure—portal pressure to superior mesenteric blood flow (ml/min×100 g small intestinal tissue) Resistance is expressed in peripheral resistance units (PRU 100=1 mmHg/ml/min×100 g). The weight of the liver and small intestine including the mesenteric lymph nodes was determined after each experiment and the other extirpated organs were also weighed Values are expressed as means ± S.E The significance of differences between sets of data was assessed by Student's *t* test

RESULTS

1 *Basal conditions and infusion of isoprenaline*

The resting hepatic arterial blood flow in the acutely denervated liver as determined in 20 cats was 18–37 ml/min×100 g liver (mean 26 0±2.5), the regional vascular resistance varying between 1 9-4.8 PRU_{100} (mean 3 6±0 2) The corresponding values for superior mesenteric flow and resistance were 22–49 ml/min×100 g intestine (mean 34 0±2 1) and 1 9-4 6 PRU_{100} (mean 3 1±0.3) respectively The blood flow levels at the end of the experiments often lasting for 2–3 h did not differ significantly from these figures neither in the liver nor in the small intestine ($P>0.1$)

Infusion in the hepatic artery of supramaximal doses of isoprenaline increased hepatic arterial

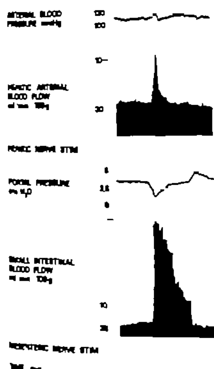


Fig. 4. Cat, 3.1 kg. The effects of synchronous stimulation (8 Hz, 5 ms, 12 V) of hepatic and mesenteric nerve stimulation on systemic arterial blood pressure, hepatic arterial blood flow, portal pressure and small intestinal blood flow. Note particularly that the portal pressure returns to the prestimulatory level during the stimulation.

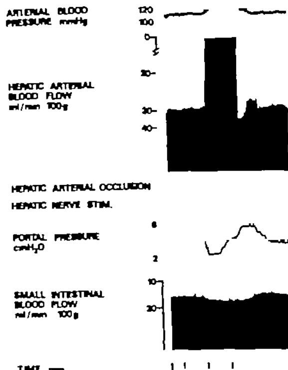


Fig. 5. Cat 2.9 kg. The effects of hepatic nerve stimulation (8 Hz 5mV 12 V) during hepatic arterial occlusion. Note the increase of portal pressure on stimulation during occlusion and the overshoot when the occlusion is released.

markedly during stimulation of the mesenteric nerves. Although this increase was partly due to an increase in mean arterial pressure there was a clear and statistically significant decrease in PRU_{ves} ($P<0.05$).

C. *Simultaneous stimulation of the hepatic and mesenteric sympathetic nerves.* The effect of simultaneous stimulation of the two vasoconstrictor fibre sets on hepatic and small intestinal blood flow was largely the same as when the nerve fibres were stimulated separately (Fig. 4). The portal pressure response differed however. Although there was in most experiments an initial pressure reduction, a slight initial pressure increase, amounting to at the most 2 cm H_2O, was sometimes observed. Irrespective of the quality and quantity of these initial changes the portal pressure approached the control level on sustained stimulation. Thus, during the steady state the portal pressure did not differ from the prestimulatory level.

III. *The effects of regional arterial occlusions and sympathetic nerve stimulation*

On occlusion of the hepatic artery systemic blood pressure increased with 10–12 mmHg and portal pressure decreased somewhat. This decrease, amounting to about 22% of control level, was well maintained throughout the occlusion period. The superior mesenteric vascular resistance remained unchanged, however. When the hepatic artery was clamped, hepatic nerve stimulation invariably caused an increase in portal pressure. This increase was quantitatively of the same magnitude as that observed during intact hepatic arterial circulation (Fig. 5). On release of the occlusion there was a shortlasting hyperemia and a concomitant further increase of the portal pressure. Occlusion of the superior mesenteric artery increased mean arterial blood pressure to the same extent as did hepatic arterial occlusion. Concomitantly there was an increase in hepatic arterial blood flow of about 8

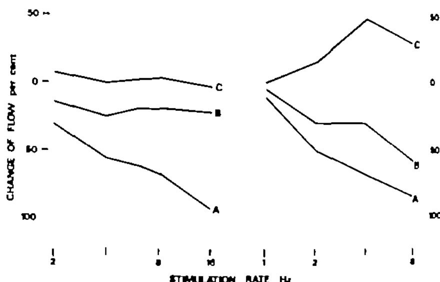


Fig 3 Frequency response for the hepatic arterial (left panel) and small intestinal blood flow (right panel) The effects of graded vasoconstrictor activation are expressed in per cent of prestimulatory blood flow levels. Note that the peak response (A) is rate dependent in both vascular beds that the steady state (B) response is rate dependent only in the small intestine and that the immediate post-stimulatory flow i e the reactive hyperemia (C) i most pronounced in the small intestine

within 1–2 min a new and well maintained flow level (steady state) was established which was in most experiments slightly below control level In some animals the blood flow even reached the control level during the stimulation The blood flow during steady state did not differ significantly at different stimulation frequencies ($P>0.2$) On cessation of stimulation a shortlasting hyperemia (30–60 s) was occasionally seen but was unrelated to the rate of the stimulation

Corresponding to the peak resistance response there was a considerable but transient rise of the portal pressure which at high frequencies of stimulation amounted to 100–200 % The portal pressure then slowly declined however and after 2–3 min stimulation a steady state level was established at about 30 % above the control level This level was well maintained throughout the stimulation The differences between the peak response and the steady state level was statistically significant ($P<0.01$) The superior mesenteric arterial blood flow e g the portal blood flow increased somewhat The superior mesenteric vascular resistance remained unchanged ($P>0.2$) however indicating that this blood flow increase was related to the concomitant increase in mean arterial blood pressure regularly observed during the stimulation

B *The mesenteric sympathetic nerves* Stimulation of the vasoconstrictor fibres to the small intestine evoked the same initial effect on the intestinal blood flow as did hepatic nerve stimulation on hepatic blood flow The threshold frequency was in most experiments 0.5 Hz After an initial peak response the resistance to flow decreased again, and was within 2–3 min followed by a sustained but moderately reduced blood flow (Fig 2 right panel). The decline towards control level was somewhat slower than that observed during stimulation of the hepatic plexus As is shown in Fig 3 right panel the peak response showed a linear correlation to the log stimulation frequency ($r=0.68$) Maximum decrease was in most experiments obtained at 8 Hz In contrast to the observations in the hepatic vascular circuit there was a more pronounced reduction of the blood flow during steady state on high (8 Hz) than on low (2 Hz) stimulation rates ($P<0.001$), and at high rates the initial blood flow reduction in some experiments remained almost unchanged throughout the stimulation Following cessation, there was a marked hyperemia lasting for several minutes Simultaneous to the peak resistance response there was a moderate decrease of the portal pressure amounting to about 24 % This initial rapid decrease was followed by a slight increase towards control level Concomitant to the hyperemia following cessation of stimulation there was a transient increase of the portal pressure amounting to about 18 %

The hepatic arterial blood flow also increased

1967), implying that β-receptor stimulation plays a minor role in the nervous response

The almost complete recovery of the hepatic arterial blood flow after the initial peak reduction would imply that the hepatic arteries might have a high sensitivity to local metabolic factors. This is also supported by the observation that occlusion of the superior mesenteric artery was followed by a decreased hepatic arterial vascular resistance It is also possible that the sympathetic nerves have a direct trophic influence on the parenchymal cells of the liver Redistribution of arterial blood flow which might explain the escape phenomenon in the small intestine (Folkow Lundgren & Wallentin 1963) appears to be a less probable mechanism in the liver (Greenway & Oshiro 1972)

The marked increase of the portal vein pressure in hepatic sympathetic nerve stimulation has been demonstrated earlier (Greenway et al 1967 Sadiq Bolhaker & Engqvist 197? Henrich & Biester 1973 Ulrich & Rodbard 1974 Lautt 1977) The pronounced increase of portal vein pressure obtained on stimulation even when the hepatic arterial supply was occluded speak in favour of a direct vasoconstrictor effect on the portal vascular bed.

Greenway et al. (1967) showed evidence that the portal vein pressure increase was well maintained throughout the stimulation despite the decline of hepatic vascular resistance towards resting values This is in contrast to the findings in the present study showing a regular decline of the portal pressure on sustained stimulation Since the decrease appears later than the blood flow increase it can hardly be secondary to changes in the portal blood flow such did in fact increase as reflected in an increased superior mesenteric flow Since an increase in portal blood flow would increase portal pressure the decline due to escape from vasoconstrictor fibre influence was even somewhat masked. The explanation for this escape phenomenon is not clear However its late appearance as compared with that occurring in the hepatic arterial circuit would make accumulation of metabolic factors possible

It has been previously shown that the reactions of the vascular bed of the stomach and colon to sympathetic nerve activation seems to be similar to those observed in the small intestine (Folkow & Neil 1971) Thus the tissue volume in all parts of the gastrointestinal tract, as recorded by plethysmographic technique, remains constant throughout sympathetic vasoconstrictor activation implying that mean hydrostatic capillary pressure is kept unchanged It has been suggested that such a haemodynamic arrangement serves to protect the gut mucosa from a massive oedema that would inevitably develop if the isovolumetric state was changed into a continuous outward filtration Considering the big capillary surface of the intestine any increase of resistance in the portal vascular bed during a generalized increase of sympathetic discharge would be potentially deleterious The result of the present study clearly shows that the portal flow resistance which makes up part of the post capillary resistance for the intestinal vascular bed is not increased when the hepatic vessels are exposed to the same constrictor fibre discharge as the intestinal vessel

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ml/min×100 g corresponding to a 30% increase PRU decreased 20% indicating that this increase in hepatic arterial blood flow was partly caused by a decrease of hepatic vascular resistance After cessation of the occlusion there was a profound hyperemia in the small intestinal vascular bed lasting for about 2 min On superior mesenteric artery occlusion the decrease in portal pressure was marked amounting to about 70% Hepatic nerve stimulation during occlusion caused a slight increase of portal pressure amounting to at the most 1 cm H_2O This was not the case when the mesenteric nerves were stimulated Simultaneous occlusion of the hepatic and superior mesenteric arteries lowered portal pressure to 0 4–0 7 cm H_2O When both these arterial supplies were clamped hepatic nerve stimulation increased portal pressure with about 0 5 cm H O On release of the occlusion a marked hyperemia was observed in both vascular beds The post occlusion hyperemia was of the same magnitude and duration as that observed after separate occlusion of the circuits

DISCUSSION

The present observation that occlusion of the hepatic artery does not affect portal blood flow is consistent with previous findings (Kock et al 1972 Krarup & Larsen 1974) During hepatic artery occlusion there was a well maintained reduction of portal pressure amounting to about 20% compared to a reduction of about 70% when the superior mesenteric artery was clamped These findings would imply that small intestinal blood flow contributes more to maintenance of the portal pressure than the hepatic arterial blood flow but also that there might exist an efficient pressure reduction between the high and low pressure systems It might even be so that the reduction of portal pressure obtained during hepatic artery occlusion is not entirely caused by a direct influence of hepatic arterial pressure on the portal vascular bed but might at least partly be due to a decreased tone in presinusoidal sphincters

Occlusion of mesenteric blood flow however caused a significant increase in hepatic arterial flow partly caused by a decrease in hepatic vascular resistance This decrease in resistance might probably be due to hepatic hypoxia as has been suggested by Kock et al (1972) Occlusion of both vascular supplies lowered portal pressure to the critical closing pressure" of the postsinusoidal veins In the present study this pressure was lower than that reported in studies on dogs however (Mitzner 1974) This difference might be explained by the fact that canine postsinusoidal veins have a more pronounced potential for sphincteric action than those of the cat (Ungváry & Donath 1969)

Vasoconstrictor fiber stimulation caused an initial peak blood flow reduction in both vascular beds This peak response correlated in a linear fashion to the logarithm of the stimulation rate Despite continuous stimulation the peak resistance response was transient and followed by a second phase of less pronounced vasoconstriction The escape phenomenon differed in some important respects in the two vascular circuits however Thus the hepatic vascular resistance regularly subsided within 1–2 min and hepatic arterial blood flow reached a new steady state which was only about 20% below the control blood flow irrespective of stimulation frequency By contrast the peak resistance response in the small intestine was more prolonged and a steady state level was as a rule not attained until 2–3 min The escape phenomenon was not observed in all animals an observation which is in accordance with earlier findings (Folkow & Neil 1971) Moreover in contrast to what we observed in the hepatic vascular bed the magnitude of the "steady state" blood flow was dependent on the stimulation rates being lower at high discharge rates Moreover poststimulatory hyperemia was more pronounced and regular in the small intestine than in the liver

The mechanisms responsible for the autoregulatory escape are still obscure In the intestine this escape phenomenon has been demonstrated both in the cat in the dog (Greenway & Oshiro 1972) and in man (Hultén Lindhagen & Lundgren 1977) but appears to be absent in the liver of the dog (Greenway & Oshiro 1972 Richardsson & Withrington 1977*a*) A similar phasic change of hepatic and intestinal blood flow has been described during infusion of noradrenaline however (Greenway et al 1967 Ross 1971 Schehadeh Price & Jacobson 1969 Richardson & Withrington 1977*b*)

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Blood flow changes in the duck during thermal panting

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BECH, C. & JOHANSEN, K. Blood flow changes in the duck during thermal panting. Acta Physiol Scand 1980, 110: 351–355. Received 14 Febr. 1980. ISSN 0001-6772. Department of Zoophysiology, University of Aarhus, Denmark.

Carotid and sciatic blood flow have been measured in resting and panting Pekin ducks using electromagnetic flowmeters. Panting induced by high ambient temperature caused the carotid blood flow to increase from 9.6 to 76.0 ml min⁻¹, while the sciatic flow declined slightly from 41.6 to 38.1 ml min⁻¹. During panting breathing rate increased 10–20 times but there were no significant changes in heart rate and mean arterial blood pressure. The carotid peripheral resistance was therefore greatly reduced, whereas sciatic resistance remained unchanged or increased slightly. The vascular beds perfused by the sciatic (legs) and carotid (upper respiratory tract) arteries are both important for heat dissipation. This study shows that when heat dissipation from the naked legs becomes inefficient due to high ambient temperature, blood flow in the sciatic tended to decline while conversely panting was associated with a large increase in carotid flow.

Key words: Hyperthermia, heat dissipation, panting, Pekin duck, regional blood flow

Lacking sweat glands birds must depend on heat dissipation by evaporation from the respiratory tract when increasing environmental temperatures diminish or reverse the skin to ambient temperature gradient. Typically this respiratory heat loss by evaporation is greatly increased with onset of panting (Richards 1970). Optimising heat dissipation during panting depends on 3 main factors: (1) The increased ventilation associated with the 10 to 30 increase in breathing rate. (2) Passage of exhaled air across the largest possible surface area of the upper respiratory tract, and (3) An increase in blood flow to the evaporating surfaces conveying heat from the body core to the sites of heat dissipation.

The magnitude and pattern of the ventilatory changes during panting have been well studied for many species (Richard 1970, Lasiewski 1972). The nature of blood flow changes to evaporating surfaces in panting birds has, however, to our knowledge only been studied by estimation of changes in regional blood flow distribution based on injection of radioactive microspheres (Wolfenson et al. 1978 b).

The present study seeks to establish the changes in blood flow to and vascular resistance in the head and legs of domestic ducks associated with hyperthermia and panting.

MATERIALS AND METHODS

Domestic ducks were obtained from a local supplier. They were housed indoors at room temperatures (20–22°C) and exposed to a 10 to 14 h light-dark cycle. They were fed commercial duck pills and had access to water at all times.

Surgery for blood vessel catheterizations and electromagnetic blood flow probe placements were done during general anaesthesia (equithesin, 2.5 ml·kg⁻¹ administered i.m.). The carotid arteries were exposed at the lower ventral side of the neck near the thoracic inlet. A Statham 2.0 mm electromagnetic flow probe was placed around the left carotid artery and anchored in place by means of a tubulating plastic rod attached to the flow probe and sutured to the neck musculature running parallel to the artery. The leads were extended out through a small skin incision and secured by sutures. Using a similar procedure a 3.0 mm flow probe was also placed around the left sciatic artery. This artery was exposed by dissection through the M. iliotibialis. A minimum of two days were allowed for post operative recovery before experimentation was started.

Calibration of the flow probes were done in situ. After termination of experiments the ducks were given a large dose of heparin and killed with an overdose of anesthesia. The left carotid artery as well as the left sci-

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Blood flow changes in the duck during thermal panting

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BECH C & JOHANSEN K Blood flow changes in the duck during thermal panting. Acta Physiol Scand 1980 110 351 355 Received 14 Febr 1980 ISSN 0001-6772 Department of Zoophysiology University of Aarhus, Denmark.

Carotid and sciatic blood flow have been measured in resting and panting Pekin ducks using electromagnetic flowmeters. Panting induced by high ambient temperature caused the carotid blood flow to increase from 9 6 to 26 0 ml min while the sciatic flow declined slightly from 41.6 to 38 1 ml min During panting breathing rate increased 10–20 times but there were no significant changes in heart rate and mean arterial blood pressure The carotid peripheral resistance was therefore greatly reduced whereas sciatic resistance remained unchanged or increased slightly The vascular bed perfused by the sciatic (legs) and carotid (upper respiratory tract) arteries are both important for heat dissipation This study shows that when heat dissipation from the naked legs becomes inefficient due to high ambient temperature, blood flow in the sciatic tended to decline while conversely panting was associated with a large increase in carotid flow

Key words. Hyperthermia, heat dissipation panting, Pekin duck regional blood flow

Lacking sweat glands birds must depend on heat dissipation by evaporation from the respiratory tract when increasing environmental temperatures diminish or reverse the skin to ambient temperature gradient. Typically this respiratory heat loss by evaporation is greatly increased with onset of panting (Richards 1970) Optimizing heat dissipation during panting depends on 3 main factors (1) The increased ventilation associated with the 10 to 30 increase in breathing rate (2) Passage of expired air across the largest possible surface area of the upper respiratory tract, and (3) An increase in blood flow to the evaporating surfaces conveying heat from the body core to the sites of heat dissipation.

The magnitude and pattern of the ventilatory changes during panting have been well studied for many species (Richards 1970 Lasiewski 1972) The nature of blood flow changes to evaporating surfaces in panting birds has, however to our knowledge only been studied by estimation of changes in regional blood flow distribution based on injection of radioactive microspheres (Wolfenson et al. 1978 b).

The present study seeks to establish the changes in blood flow to and vascular resistance in the head and legs of domestic ducks associated with hyperthermia and panting.

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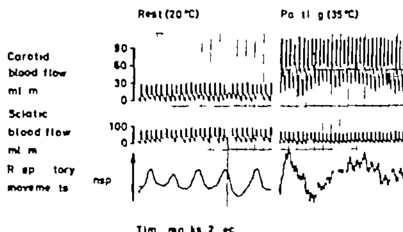


Fig 1 Recordings of unilateral carotid and sciatic blood flow measured at thermoneutral condition (ambient temperature 20°C) and after 1 hour continued panting at ambient temperature of 35°C Also shown are the breathing movements depicting the increase in respiratory frequency during panting.

atic artery were cannulated on both sides of the flow probes with polyethylene tubings (PE 60) Subsequently the arteries were perfused with a physiological salt solution using a Harvard peristaltic pump calibrated volumetrically In this way the flow probes were calibrated in the same position as during the experiments

Blood flow was measured by Statham electromagnetic flow meters (model SP 2202) and mean and pulsatile values recorded on a 6-channel Brush recorder (Mark 60)

Catheters for blood pressure recording were implanted via the brachial artery The catheter was flushed daily with heparinized saline Arterial blood pressures were measured using Statham pressure transducers (model P23d) The pressures were recorded on the 6-channel Brush recorder

Cloacal and ambient temperatures were recorded by an Ellab potentiometer and displayed on a Beckman recorder (model 511 A) together with the output from a mercury strain gauge placed around the chest of the ducks and used for monitoring breathing rate

During experimentation the ducks were placed in a small box which prevented movement but allowed normal standing and sitting postures. The box was placed in a climatic chamber where the temperature and humidity could be controlled to within ±0.5°C and 5% respectively Following -3 hours rest at 20°C and relative humidity 50% measurements of all parameters were obtained and the climatic chamber temperature was set to 35°C while retaining the relative humidity at 50% The bird started panting 45-60 minutes after resetting of the temperature Following one hour of continued panting all parameters were measured again Only measurements from periods when the birds were resting have been used in the analysis.

RESULTS

Fig 1 shows records of unilateral carotid and sciatic blood flow in a resting duck at thermoneutral conditions (20°C) and following 1 h of sustained panting at an ambient temperature of 35°C Respiratory movements giving frequency of breathing are

Table 1 *Comparison between body temperature breathing rate and circulatory variables in Pekin ducks at rest and during panting*

Mean values ± S E N S = not significant

		Resting (20°C)	Panting (35°C)	*P*
Body temperature (°C)	9	41 3±0 4	41 8±0 5	<0 05
Breathing rate (min)	9	18 7±6 1	235±71 8	<0 001
Unilateral carotid blood flow (ml min)	9	9 3± 3	26 0±9 0	<0.001
Unilateral sciatic blood flow (ml min)	5	41 6± 4 3	38 1± 4 9	N.S
Heart rate (min)	9	146 6± 4 8	137 5±13 9	N.S
Mean arterial blood pressure (mmHg)	5	135 1±11	136 5±1 5	N.S
Carotid peripheral resistance (dynes sec/cm⁵)*		11 56±10⁵	4 18±10⁵	
Sciatic peripheral resistance (dynes sec/cm⁵)*		59±10⁴	85±10⁴	

Calculated from mean values of blood flow and arterial blood pressure

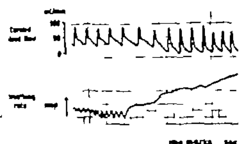


Fig. 2 Pulsatile carotid blood flow measured at high ambient temperature (35°C). The lower tracing shows the respiratory movements measured with mercury strain gauge. It evident that hen panting suddenly stops, which may happen spontaneously the carotid blood flow ...creases

...ken in the bottom tracings indicating an increase from 20 breaths per minute to 273 breaths per min associated ith panting. The conspicuous increase in carotid blood flow represents an elevation of mean flow from 9.8 ml min⁻¹ to 45.1 ml min, or a 4.6 fold increase. The magnitude of the carotid blood flow increase was variable between specimens (Table 1) as was the rate of the change in blood flow. In some experiments blood flow rose gradually ith a rise in cloacal temperature before onset of panting; in others the major blood flow increase coincided ith onset of panting. Upon termination of panting carotid blood flow typically declined abruptly (Fig. 2). Sciatic blood flow did not increase ith panting (Fig. 1 Table 1)

Both carotid and sciatic blood flow were very labile. Thus a sound stimulus like opening a door or tapping on the experimental box would cause a transient increase in both carotid and sciatic blood flow at non-panting condition (left side of Fig. 3). The lack of a sciatic blood flow increase with panting (Fig. 1) furthers the evidence that the increase of carotid blood flow with panting is a truly specific response. When a disturbing sound stimulus was applied during panting, the sciatic blood flow changed in a similar fashion as during non-panting. The greatly increased level of carotid blood flow during panting however abruptly but transiently dropped off when the sound stimulus was applied (Fig. 3 right). Following the stimulus the two flow patterns and breathing rate returned gradually to pre-disturbance values.

Table 1 summarizes results obtained in 9 expts on different specimens. Sciatic blood flow and systemic arterial pressure were measured in 5 of these expts

The rise in body temperature (cloacal temperature) from resting to panting condition usually occurred gradually from the time when the ambient temperature was set to 35°C. The rate of increase in cloacal temperature declined markedly when panting started and in most cases stabilized or even declined during the period of panting.

Table 1 shows that the changes in breathing rate carotid blood flow and carotid vascular resistance were all highly significant while changes in sciatic blood flow and resistance systemic arterial pressure and heart rate were not statistically significant The increased carotid blood flow during pant

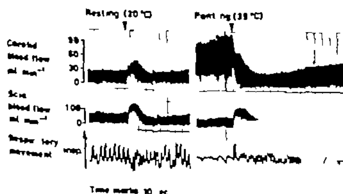


Fig. 3 Recordings of unilateral carotid and sciatic blood flow measured at thermoneutral conditions (ambient temperature 20°C) and after 1 hour continued panting at ambient temperature of 35°C. The arrows at the top mark the time when the duck was disturbed by an acoustic stimulus.

ing was always associated with an increased beak temperature

DISCUSSION

Various authors have reported on heart rate and arterial blood pressure associated with thermal panting in birds (Frankel et al 1962 Linsley & Burger 1964 Whittow et al 1964 Harrison & Biellier 1969 Smith 1972 Marder 1973 Bech & Johansen 1980) but this information is not well suited for evaluation of thermoregulatory adjustments in cardiovascular function

Wolfenson et al (1978*a* *b*) have reported on blood flow distribution in normothermic and hyperthermic hens using radioactive microspheres Their results showed panting to be associated with marked increases (×15) in blood flow to the tongue larynx and trachea

Many studies of mammals have addressed problems related to changes in regional blood flow at hyperthermic conditions (Ederstrom 1954 Hales 1973 1974 Hales & Dampney 1975 Hales 1976) One series of studies on blood flow changes to the tongue of the dog has particular relevance to the present study (Krönert & Pleschka 1976 Pleschka & Krönert 1976) These studies demonstrated an increase of 5–6 times in lingual blood flow during panting and that this blood flow increase correlated with a greatly increased heat loss from the tongue (Pleschka & Krönert 1976)

The thermoregulatory significance of the directly measured increase in carotid flow presently recorded in panting ducks cannot yet be evaluated nor is it known how much of the blood flow to the head is distributed to the upper respiratory tract during panting Based on measurements of respiratory water loss and cloacal and surface temperatures Menuam & Richards (1975) concluded that the nasal buccal and upper tracheal surfaces in the fowl were the most important sites of evaporative heat dissipation

Cardiac output in resting ducks at thermoneutral conditions has been reported by several authors (Sturkie 1966 Folkow et al 1967 Jones & Holeton 1972*a* *b* Scheid et al 1978) Taking a mean value for these studies of 3?9 ml min kg as a reference value for cardiac output in our study carotid blood flow at thermoneutrality will represent 2 3 % of cardiac output The figure for sciatic flow will be 10.2 % The relatively large fraction of cardiac output directed through the sciatic arteries in birds has been related to the important heat dissipating role of the naked feet (Jones & Johansen 1972). The only published value for cardiac outputs at thermoneutral and panting conditions in a bird appears to be a 6 % increase with panting reported for the mute swan (*Cygnus olor*) (Bech & Johansen 1980). We have stipulated cardiac output to increase by 10 in the duck during panting. The fractional distribution of the cardiac output to the carotid and sciatic arteries will then be altered from 2.3 % to 5.4 (carotid) and from 10.2 % to 8.5 % (sciatic) for the resting and panting conditions respectively Mean systemic arterial pressure remained stable with onset of panting implying an unaltered driving force through the two vascular beds compared. The peripheral vascular resistance must hence have changed in accordance with the altered flow rates decreasing from 11.56×10^5 to 4.18×10^5 dynes sec cm^{-5} for the carotid while the sciatic resistance increased insignificantly from 2.59×10 to 2.85×10^5 dynes sec cm^{-5} (Table 1) The non-panting values for vascular resistance compare favourably with values reported by Butler & Jones (1971) on the duck

An opposite change of vascular resistances between vascular beds serving different functions is commonplace also when one of the vascular beds serves as an effector mechanism in thermoregulation Thus skin vascular resistance declines while intestinal vascular resistance increases in mammals during heat stress (Schönung et al. 1971 Simon 1971) We are unaware however that two vascular beds both important in thermoregulation, such as the sciatic and carotid beds in ducks, have been demonstrated to show a decrease in vascular resistance (the carotid) and an unchanged resistance (the sciatic) during ambient thermal stress.

Baudinette et al (1976) working on the herring gull documented that 37–56 % of total heat production at rest was lost via the feet while during flight when metabolic heat production was estimated to be increased 7 times approximately 80 % of the total heat production was dissipated across the extremities During flight blood flow increased about 4 times through the sciatic artery When comparing these data to ours on the panting duck one must remember that panting is associated with only a slightly higher heat production (Bouverot et al. 1974) Moreover heat dissipation by convection and conduction becomes less important due to the

[illegible]hered surface to ambient temperature gradients the practical problem for the panting bird at rest is [illegible] to dissipate more heat, but to alter the modes of [illegible] dissipation from conduction con ection and [illegible]diation, to that of evaporation This change of [illegible] energy requires an altered blood flow and thus [illegible] transport favouring the evaporative surfaces [illegible] there is nothing is gained by an increased perfu[illegible] to sites principally involved in heat dissipation [illegible] convection and conduction.

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Relation between cell length and force production in urinary bladder smooth muscle

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Key ords Urinary bladder cell length length–tension relations cell force

In vertebrate smooth muscles the maximum force developed per unit sectional area can equal or even exceed that of a skeletal muscle (Murphy 1976, Mulvany & Halpern 1976, Gabella 1976*b*) In spite of their relatively low myosin content (Murphy Herlihy & Megerman 1974) The number of studies in this field is small The taenia col of the guinea-pig and some arterial muscles are the tissues most commonly investigated It would clearly be interesting to assess the force development in other smooth muscles, particularly in those in which there is a different geometrical arrangement of muscle cell than in taenia coli or arterial media.

Smooth muscles consist of cells that are coupled to each other in a complex manner (see Gabella 1977) In order to analyze the results of experiments on muscle strips in terms of behaviour of the individual cells it is necessary to know how the length of the cells varies with the length of the preparation. In earlier studies (Cooke & Fay 1972, Uvelius 1976 Dreke & Murphy 1978) smooth muscles stretched to different lengths were macerated with nitric acid or KOH and the length of individual isolated muscle cells were measured. These studies suggest that the cells are essentially coupled in series that is the changes in cell length mirror the changes in length of the muscle strip A limitation of the method used is that the length of the cells may be affected by the maceration Halpern Mulvany & Warshaw (1978) measured with interference microscopy stretch-induced changes in the distance between intracellular markers in muscle cells of small mesenteric arteries in vitro They found a linear relationship between the changes in the separation of the intracellular markers and the circumference of the vessel It remains to be established however whether the same holds true for the tapering parts of the cell

With the present experiments we aimed to measure the maximum force generated by the detrusor muscle of the bladder (a muscle which can be set up in vivo without direct surgical interference with the tissue) In addition by applying morphometric techniques in light and electron microscopy we wanted to calculate the force generated

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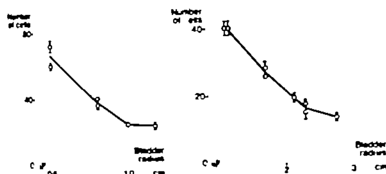


Fig. 1. Number of muscle cells across the bladder wall vs. outer radius for guinea-pig (left) and rabbit (right) bladders. Mean ± S.E. (25 determinations for each bladder). 6 guinea-pig bladders and 8 rabbit bladders were used. The number of cells across the all decreases as the bladder volume is increased except for the highest volumes.

...dius, ...hen dealing ...th the circular musculature ...s the radius of the fluid in the bladder assuming spherical ...try form. All data are ...xpressed as mean ± standard ...ror (S.E.).

RESULTS

... Thickness of the muscle coat and number of cells

The thickness of the wall of the bladder and that of its muscle coat decreased with the distension of the organ. With an increase in the bladder volume and corresponding distension of the wall the bundles of muscle cells spread tangentially, the transverse sectional profiles of the bundles tended to be round in empty bladders whereas they tended to flatten in distended bladders (Plate 1). As a consequence of this re-arrangement the number of muscle cells across the thickness of the wall (see Methods) decreased when the organ is inflated (Fig. 1). In other words, with distension of the organ, the muscle coat becomes thinner and, at any one point, is formed by a smaller number of cells. With distension of the bladder the profiles of individual muscle cells (in transverse section) became smaller in area. This change is borne out by the electron micrographs (Plate) and was expressed in a quantitative way by counting the number of muscle cell profiles per unit sectional area of muscle coat (cell packing density). Muscle bundles which were in exact transverse section (as confirmed by cutting a series of sections) were used. I... the guinea-pig the packing density ranged from 36000 cells per mm² in nearly empty bladders to 170000 cells per mm² in distended bladders (Fig. 2). In the rabbit at 5 ml bladder volume the packing density in two experiments was 86000 and 64000 per mm² respectively. At 20 ml 117000 and 141000 cells per mm² were counted in two experiments.

Lengths of smooth muscl... cells

The approximate length of the muscle cells was estimated with a technique (see Methods) based on measurements on longitudinal and transverse sections from adjacent areas of the same tissue block. The results show a correlation between radius of the bladder and muscle cell length (Fig. 3) in both guinea-pig and rabbit. The range is 160 to 580 microns (corresponding to radii of 0.4 cm and 1.2 cm)

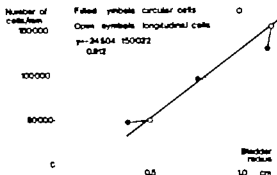


Fig. 2. Packing density of muscle cells. Guinea-pig. Number of cross sectioned longitudinal and circular muscle cells per mm² vs. bladder inner (circular muscle) and outer radius (longitudinal muscle). Filled symbols indicate circular muscle and open symbols longitudinal. Symbols connected by broken lines indicate determinations from the same bladder. The best fitting straight line by linear regression analysis for the entire material is shown. 6 bladders were used. Each point is based on 184–405 cells.

by the individual intact muscle cells in situ Direct measurements of force in single muscle cells have been made by Fay (1977) on muscle cells enzymatically isolated from the toad stomach

METHODS

Material

Adult guinea-pigs (body weight 300–500 g) and rabbits (body weight 2 500–3 500 g) were used

Histology

For the structural studies the animal was stunned and bled the abdomen was opened longitudinally from pubis to sternum and the urinary bladder exposed In some experiments the urethra was ligated to prevent emptying of the urine inside the bladder: the bladder was then dissected out immersed for 10 min in oxygenated Krebs solution at about 30°C and fixed The volume of the lumen was measured as the volume of urine collected when the bladder was slit open after about 15 min in fixative The above procedure gave poor preservation of the fine structure of muscle cells and was therefore modified by replacing the urine with Krebs solution through a cannula inserted into the urethra before excision of the bladder The emptying of the bladder was carried out in small steps and the refilling was carried out through a gravity fall not greater than 10 cm of water The highest degrees of bladder filling required up to 30 min to become complete The bladder was then fixed by transferring it from Krebs solution to the fixative solution The latter was at room temperature and consisted of 5% glutaraldehyde in 100 mM Na cacodylate at pH 7.4 with 0.4 mM $CaCl_2$ After about 10 min in fixative the bladder was slit open cut into identifiable portions (8 to 14 portions for each bladder) and transferred into fresh fixative The fixation procedure lasted ~6 h after which the specimens were washed for 15 min in cacodylate buffer and immersed for 60–90 min in 2% osmium tetroxide in cacodylate buffer The specimens were then block stained in a 1% aqueous solution of uranyl acetate for 30 min at room temperature and dehydrated in graded ethanols and epoxypropane and infiltrated in Araldite Sections for light microscopy (1.5–3.5 µm thick) were cut with glass knives collected on microscope slides examined and photographed unstained in a phase contrast microscope Sections for electron microscopy cut with glass knives were collected on copper grid stained with lead citrate and examined in a Philips 300 electron microscope All the histological observations were carried out on a region of the bladder equidistant from the upper and the lower poles (equatorial region) and particularly from the ventral part

Morphometry

Measurements of the thickness of the muscle coat were carried out on photographic prints or directly in the microscope with the aid of a calibrated eye-piece The numbers of muscle cells present across the full thickness of the muscle coat were counted by counting the numbers of muscle cells intersected by radial lines drawn orthogonally through the wall The transverse sectional area of the muscle bundles was measured with the aid of a planimeter on photographic prints and the number of muscle cell profiles within a bundle counted with the aid of a cell counter The average length of smooth muscle cells estimated with the method described in detail elsewhere (Gabella 1976a). The method is based on counting the percentage of nucleated cell profiles in low power electron micrographs of a transversely sectioned bundle and measuring the length of nuclei in longitudinal sections of an adjacent portion of the same muscle bundle examined in light microscopy The number of cells counted for each determination ranged between 448 and 25? (mean 1?) and the number of nuclei between 76 and 115 We have not made corrections for the shrinkage of the tissue and compression of the sections In smooth muscles these factors introduce only a small error in the assessments of areas and lengths, and do not affect our results which are comparisons between uniformly processed tissues.

Pressure-volume experiments

The experiments were carried out on ether-anesthetized guinea pigs weighing about 400 g. A mid ventral section ? cm long exposed the bladder which was then cannulated through the urethra The bladder was emptied and tied leaving the cannula in place After ligation the ureters were cut off at 0.5–1 cm distance from the bladder and were drawn together with adjoining tissue including the pelvic nerve through a 0.5 cm long plastic tube with a platinum electrode at each end The electrodes from each side were connected to a Grass S44 stimulator via a Grass SIU5 stimulus isolation unit The cannula was connected to a Statham P23Dc pressure transducer placed at the same level as the bladder Through a branch tube the bladder was filled with Krebs solution by gravity to desired volumes. After the pressure had stabilized the nerve supply of the bladder was supramaximally stimulated on both sides by a train of square waves (usually 30 V 2 ms duration 30 Hz) for 15 s This period was long enough to allow the bladder to produce a pressure plateau The bladder was then filled to a different volume and the sequence was repeated When the pressure for the highest desired volume had been obtained the bladder was emptied in steps and stimulated again at each volume The time between each stimulation period was 10 min Repeated stimulations under these conditions, but with constant bladder volume gave almost identical pressure values for more than one hour

For sake of simplicity we have treated the urinary bladder as spherical in shape In the guinea-pig the bladder is practically iso-diametrical at all degrees of distension On the other hand the bladder of the rabbit becomes, with extensive distension slightly elongated along an axis from the upper (cranial) to the lower (caudal) pole When dealing with the longitudinal musculature (which is located closer to the exterior than to the interior of the bladder wall) the radius of the bladder is expressed as bladder outer radius This is obtained by adding the volume of the bladder tissue to the fluid volume in the bladder and calculating the radius for this total volume In other experiments bladders were weighed In guinea-pigs bladders weigh about 0.3 g and thus have a volume of about 0.3 ml Rabbit bladders have a weight of about 1.8 g. The inner

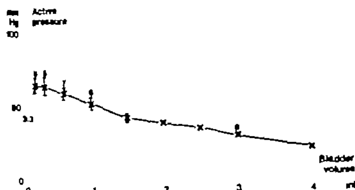


Fig. 4. In vivo experiments of bilateral pelvic nerve stimulation. Active pressure vs. bladder inner volume. Guinea-pig. 4 animals were used. Mean ± S.E. Number of determinations indicated in the figure. The maximum active pressure is achieved at a bladder volume of 0.15 ml.

[...] section 4) by this plane and from the values of [ce]ll length as function of bladder volume (or [...]) (Fig. 3) the length-tension relation of single [mu]scle cells can be calculated (Fig. 6). The [ma]ximum active tension per cell is about 5.5 μN [...]ing the highest and the lowest estimates of the [tot]al number of cells (section 4) the maximum ten[sio]n per cell ranges between 5.0 and 6.0 μN.

[DI]SCUSSION

[I]n this study we combined a morphological and a [ph]ysiological method to study the generation of [for]ce in the detrusor muscle of the bladder. With increase in volume of the bladder the wall is distended and the muscle coat becomes thinner. At the same time the muscle cells become thinner and their length increases. The morphometric study indicates that over most of the wide volume range investigated there is a linear relation between bladder radius and muscle cell length. It is therefore unlikely that there is any significant longitudinal slippage or sliding of the cells past each other when the bladder is distended, a result which is in agreement with previous observations on rabbit urinary bladder (Uvelius 1976) and on other smooth muscles (Cooke & Fay 1977, Driska & Murphy 1978). The relation between cell length and bladder radius de-

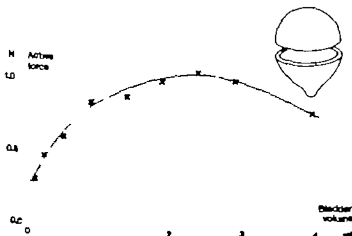


Fig. 5. Force produced by the longitudinal musculature holding the two indicated hemispheres together vs. bladder inner volume. The force developed is maximal at bladder volume of 2.5 ml.

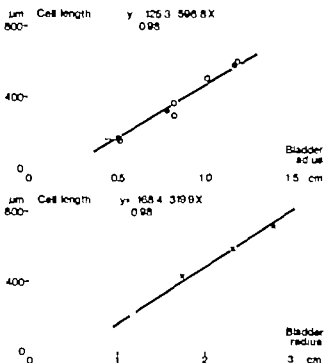


Fig 3 Estimated cell length of smooth muscle cells vs inner radius (circular muscle cells) or outer radius (longitudinal muscle cells) Upper diagram guinea-pig six bladders Filled symbols indicate circular and open symbols longitudinal muscle Connected symbols indicate determinations from the same bladder Lower diagram Rabbit eight bladders. Longitudinal muscle The best fitting lines obtained by linear regression analysis are shown

in the guinea-pig and 200 to 700 (radii of 1 1 cm and 2 8 cm) in the rabbit

3 *Volume–pressure relation*

In the guinea-pig the maximum active pressure following pelvic nerve stimulation amounted to about 65±5 mmHg and it was reached at a bladder volume of 0 15 ml The pressure developed upon stimulation steadily declined with greater distension of the organs reaching a value of about 30% of maximum when the volume of the bladder was 4 ml (Fig 4) At all bladder volumes examined the passive pressure was small compared to the active pressure at a bladder volume of 4 ml it amounted to about 3 mmHg

4 *Force developed by the bladder wall*

The relation between pressure in the lumen and force developed by the wall of the organ is expressed by the equation $F = r^2 \times \pi \times P$ where F is the force in Newton P is pressure in Pascal and r is the radius of the spherical organ (F being the force trying to separate the two hemispheres) The force developed inside the wall to antagonize the effect of the lumenal pressure obtained during contraction is produced by the bundles of smooth muscle holding the two hemispheres together This force is expressed by the equation $F_b = A \times T$ where A is the area of cross sectioned muscle and T is the tension produced per unit area of this muscle If the volume of the bladder is maintained constant as in the present expts $F = F_b$ From the above equation and by using the pressure values of Fig. 4 the active force developed by the wall of the bladder following nerve stimulation has been calculated as a function of the volume of the bladder (Fig 5) The maximal force holding the two hemispheres together is 1 04 N and is attained at about 3 5 ml bladder volume

Some uncertainty in this calculation is introduced by the measurements of the transverse sectional area of the "longitudinal" musculature slightly obliquely sectioned muscle bundles whose "longitudinal" pull is less effective than that of transversely sectioned bundles were included whereas highly obliquely sectioned bundles (which must still contribute some force in the longitudinal direction) were excluded With this source of uncertainty in mind we refer to the cells which effectively pull the two hemispheres together being "approximately in transverse section" In order to calculate the force developed by a single muscle cell from the tension produced by the whole bladder (see section 5) an estimate of the total number of cells pulling the two hemispheres together was needed The total number of muscle cells which were approximately in transverse section in an equatorial plane of the guinea pig bladder at various volumes (calculated from the cell packing density the total sectional area of the muscle coat and the percentage of approximately transversely sectioned bundles) was 185 000 (mean of 6 determinations S E = 24 000) The scatter is high and there is a tendency towards an increase in number of cells with increase in bladder volume We consider this trend as reflecting the uncertainty and inaccuracy of the counting methods

5 *Maximum active tension per cell*

If we consider an equatorial plane through the guinea-pig bladder the force generated at various bladder volumes can be calculated from the tension produced by the "longitudinal" muscle pulling the two hemispheres together From the number of cells approximately transversely sectioned (185 000,



...re 2. Electron micrograph of the musculature of guinea pig bladder with content volume of 2.0 ml. A bundle of ...scle cells in transverse section (to the left) and one in highly oblique section (to the right). Bar 10 μm

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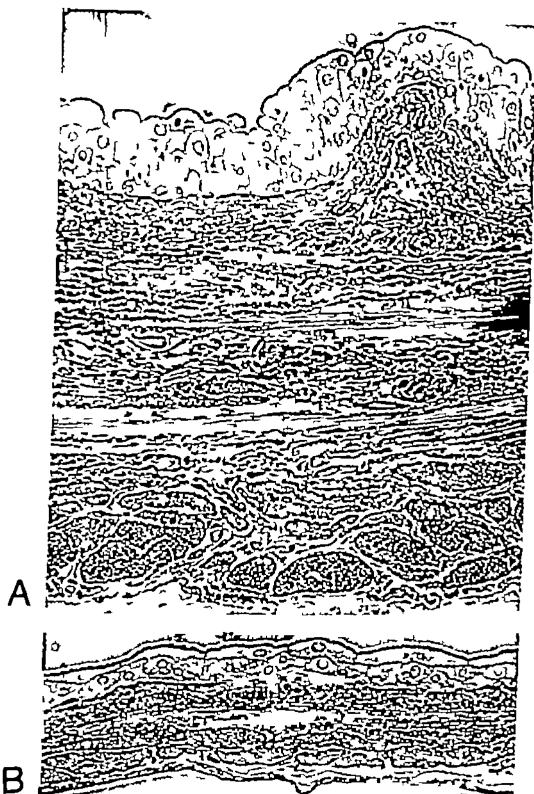


Plate I Phase contrast micrographs of the wall of the rabbit urinary bladder (full thickness) In A the content volume was 5 ml in B it was 40 ml The epithelium is at the top i both micrograph Bar 100 μm

viates from this linearity at very small bladder vol umes but here the thickness of the bladder wall is very large compared with the volume of the lumen and probably a more complex structural model should be used (one probably involving different activity in concentric layers of musculature). Estimates of cell volume were not carried out but the measurements of packing density of muscle cells



Fig. 2. Electron micrograph of the musculature of guinea pig bladder with a content volume of 2.8 ml. A bundle of muscle cells in transverse section (to the left) and one in highly oblique section (to the right). Bar 10 μm.

per and transverse sectional area indicate a linear relation between bladder radius and cell packing density (cells/mm²). Distension of the wall is thus accompanied by a proportional increase in length of the individual muscle cells and the elongated muscle cells have a proportionally thinner transverse sectional profile and therefore an increased packing density. Two other structural changes in the muscle

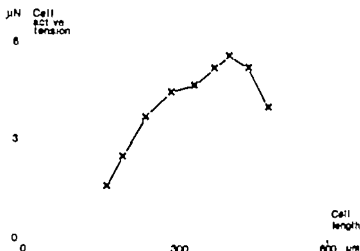


Fig 6 Calculated length–tension relation for the average individual longitudinal muscle cell

coat accompany the distension of the wall The transverse sectional profiles of the bundles of muscle cells tend to be round at small bladder volumes but are flattened tangentially at large bladder volumes (Plate 1) and so are the profiles of the individual muscle cells Moreover with distension of the wall there is some spatial re-organization of the bundles of muscle cells a set of changes that we do not yet understand well but which involve some lateral sliding of muscle bundles over each other

The pressure–volume relations obtained are qualitatively similar to those obtained by others (see e g Carpenter 1968 de Sy 1971) The estimates of force produced by the individual muscle cell were derived from these measurements of bladder pressure and from the histological data but they required a number of assumptions to be made The assumption that the bladder can be considered a sphere is reasonable for the guinea-pig (whereas in the rabbit the bladder is somewhat elongated from pole to pole) and the distensibility of the wall appears to be uniform throughout the organ with the exception of the relatively small region of the trigonum which is less stretchable We also assumed that—except at very small bladder volumes—all the muscle cells of the organ behaved mechanically uniformly namely that they were all of the same length and contracted to the same degree The estimate of the total number of muscle cells developing tension across the equatorial plane was somewhat uncertain because of the presence of obliquely arranged bundles of cells Moreover the estimates of *maximum* force refer strictly to the experimental conditions used (bilateral electrical nerve stimulation) and do not imply that this is the maximum absolute force that the tissue is capable of producing It is possible that under other conditions more powerful contractions may take place. The very concept of maximum force remains difficult to define for smooth muscle since it depends on the type of stimulation and the excitability and the metabolic conditions of the tissue and in a more general way on the past history of the muscle An advantage of our experimental approach was that the force measurements could be carried out without direct interference with the organ The vascular supply was intact and no cuts had to be made in the muscle

Maximum active force for guinea-pig bladder was produced at a volume of ? 5 ml (Fig 5) corresponding to an outer radius of 0 88 cm This corresponds according to Fig 3 A to a cell length of about 400 µm At this length the estimates of cell force range between 5 and 6 µN Other studies of force per cell have reported values of 5 µN in vascular smooth muscle (Mulvany & Halpern 1976) and 2.3 µN in rabbit urinary bladder (Uvelius 1976) The latter estimate is lower than the one we have obtained in the present experiments on guinea-pig bladder We think that the difference may be due to some damage inevitably produced in the dissection and mounting of the rabbit muscle strips in the earlier experiments (Uvelius 1976) On this account the present experiments are more reliable since they involve no direct interference with the organ during the measurements of force The maximum force produced per cell is comparable to those obtained by Fay (1979) on isolated muscle cells from toad

tomach In his study values from 2 μN and up- ards as obtained giving a mean value of 2.6 N/ m² when correction as made for cross sectional rea.

The number of cells per mm² amounts to 107 000 (a radius of 0.075 cm (Fig. 2). The active force per m² of cross sectional area would then be about 59 I. This value is higher than that in isolated strips of the urinary bladder (19 N×cm⁻²) (U ellus 1976) mainly on account of the more appropriate way of loading the muscle adopted in the present ex eriments. The present value of 59 N×cm⁻² is fairly lose to those obtained for the media of the pig arotid artery (37 N cm⁻²) (Murphy et al 1974) nd for the guinea-pig taenia coli (4 N×cm⁻²) (Gabella 1976b). The latter value is increased to bout 74 N cm⁻² when the force is expressed per nit area of contractile material When allowance is made for the different experimental approaches and or some uncertainty in the notion of maximum orce, it appears that the maximum amounts of orce measured vary rather little in the different muscles tested. In these arterial intestinal and urinary muscles the values are as high or higher than those calculated for striated muscle fibres (Close 1972)

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Vanadate-induced oliguria and vasoconstriction in the cat

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LARSEN, J. A. & THOMSEN, O. Ø. Vanadate-induced oliguria and vasoconstriction in the cat. Acta Physiol Scand 1980, 110: 367–374. Received ... Febr. 1980. ISSN 0001-6772. Institute of Physiology, University of Aarhus, Denmark.

The effect of sodium orthovanadate (vanadate) on kidney function and hemodynamics was tested in chloralose anesthetized, fasting cats. Vanadate given ... caused reversible marked decrease in glomerular filtration rate and urine production without significantly affecting the fractional water excretion rate. In the recovery period, after administration of vanadate, urine flow and the urinary excretion rate of electrolytes were only slightly different from control values. This action of vanadate appears to be due mainly to constriction of the renal blood vessels leading to a fall in glomerular capillary pressure. Vanadate likewise caused a dose dependent decrease in arterial conductance and blood flow of other vessels in the splanchnic area, whereas the femoral and carotid arteries were reversely and less affected. The vascular effect appears to be caused by a direct action of vanadate on vascular smooth muscle, but the mechanism of action is still unsettled.

Key words: Kidney function, vanadate, glomerular filtration rate, hemodynamics

In a previously published short communication we reported that vanadate caused a marked decrease in urine production in the cat (Larsen et al. 1979) in contrast to the diuretic effect of vanadate observed in rats (Balfour et al. 1978). The oliguric effect appeared to be due to a direct constrictive action of vanadate on the renal blood vessels leading to a fall in glomerular capillary pressure.

The present publication provides additional evidence that vanadate-induced oliguria is caused predominantly by a fall in glomerular capillary pressure. The results also demonstrate that vanadate is a potent constrictor of other vascular areas in the cat.

METHODS

Young cats weighing approx. 3.0 kg were fasted overnight and anesthetized with chloralose (50 mg kg) and an initial dose of Nembutal (30 mg). Body temperature was kept close to 38°C by external heating.

Catheters for infusions and blood sampling were placed in the femoral veins and arteries. Mean arterial blood pressure was recorded from the femoral artery by means of a Statham manometer (Elema Schönander). The abdomen was opened by a midline incision and when kidney function was followed, a catheter was placed either in the urinary bladder or in one or both ureters, and urine was collected in 5–10 min periods. When hepatic artery blood flow was measured, the common hepatic artery was dissected free and the right gastro-duodenal artery ligated. Flow was then measured in the common hepatic artery. In some experiments a catheter was placed in the portal vein via a peripheral mesenteric vein for blood pressure measurement.

Blood flow was measured by means of an electromagnetic flowmeter (Nycotron, Oslo) and the probe was placed on the vessel after removal of surrounding connective tissue and nerves. Zero adjustment was performed according to the manufacturers' manual and calibration made on the femoral artery and abdominal aorta. Pressure and flow were recorded simultaneously on a Beckman S II Dynograph.

When kidney function was examined 50 ml 0.6% NaCl was given ... at the start of the experiment in order to stimulate urine flow. Glomerular filtration rate was estimated from urinary inulin clearance. Inulin was given as priming dose of 800 µg unlabelled inulin and 1 µCi ^{14}C-inulin in 8 ml 0.9% NaCl followed by continuous infusion of 16 µg unlabelled inulin and 0.01 µCi ^{14}C-inulin in 0.1 ml 0.9% NaCl per min.

Vanadate (sodium orthovanadate) was dissolved in isotonic sodium chloride and given ... either as a single dose (1 ml in 1 min) or continuously (0.1 ml min).

When the operative procedures were finished ... re-

Table 1. *Kidney function before (A) during (B) and after (C) infusion of vanadate 0.5 μmol kg ml*
Urine was collected from the right ( ) and left (l) ureter in 2 expts and from only one ureter in experiments

	Urine flow (mg kg min)			^{14}C-inulin urine / ^{14}C-inulin plasma			^{14}C-inulin clearance (mg kg min)		
	A	B	C	A	B	C	A	B	C
1	10 3	0 9	4.3	111	177	706	1 128	145	876
1	7 1	0 7	6 1	141	177	190	990	76	836
2	5 9	0 3	4 0	283	226	41	1 663	135	940
1	4 8	0 6	4 2	45	478	227	1 166	96	924
3	5 5	0	5.6	309	556	268	1 690	111	1 484
4	4 3	0 1	10 4	393	313	130	1 679	31	1 308
Mean	6 3	0 4	5 7	47	31	200	1 386	99	1 061
S.E.	0 9	0 2	1 0	43	69	23	13	17	109
A-B	0 001<P<0 005			n.			P<0 001		
A-C				n.			0 01<P<0 02		

to 0.016±0 003 ml kg min^{-1} (mmHg)$^{-1}$ (0 01<P<0 02). The changes in blood pressure and renal blood flow are intimately related to the observed changes in urine flow.

In 4 expts the effect of vanadate (0.5 μmol kg min^{-1}) on the renal clearance of ^{14}C-inulin was examined. The results are listed in Table 1 and a typical experiment is illustrated in Fig 1. In two of the cats urine from both ureters was examined, but in two cats urine from only one ureter was used as bleeding occurred in the other. It will be noticed that vanadate induced a severe but reversible fall in ^{14}C-inulin clearance which corresponded in time to the observed changes in urine production. From Table 1 it appears that, contrary to the marked changes in ^{14}C-inulin clearance caused by vanadate there was no significant change in the urine-to-plasma ratio of ^{14}C-inulin. In these experiments slight but not significant increases in the urinary excretion rate of sodium and potassium occurred after termination of vanadate administration (0.88±0 16 to 1 44±0 49 and 1 00±0.27 to 1.55±0 44 μEq kg min respectively).

In 1 expt the effect of vanadate on ^{14}C-inulin clearance was examined after previous administration of Furosemide (1 mg kg). The results are listed in Table 2. It will be seen that as expected Furosemide caused a marked increase in urine flow and an equally marked decrease in urine-to-plasma inulin concentration ratio while the clearance of inulin was only slightly depressed. Also under these circumstances did vanadate decrease urine flow whereas the urine to-plasma ratio of ^{14}C inulin remained unaltered.

Vanadate and vascular reactions. In a series of experiments the effect of vanadate on renal hemodynamics as well as the hemodynamics of other vascular beds were further examined. Doses up to 0 1 μmol kg were without effect. 0 5 μmol kg caused an increase in mean arterial blood pressure from 129±8 to 146±7 mmHg (n=11). 1 0 μmol kg increased blood pressure from 123±10 to 145±

Table 2. *The effect on kidney function of Furosemide 1 mg kg followed by vanadate 0.5 μmol kg min*

	Urine flow (mg kg^{-1} min)		^{14}C-inulin urine / ^{14}C-inulin plasma		^{14}C-inulin clearance (mg kg min)	
	R	L	R	L	R	L
Control	3 3	3 1	381	390	1 260	1 195
Furosemide	109 4	115 0	9	9	1 030	1 001
Vanadate	0 9	0 6	5	7	5	4

X-urine

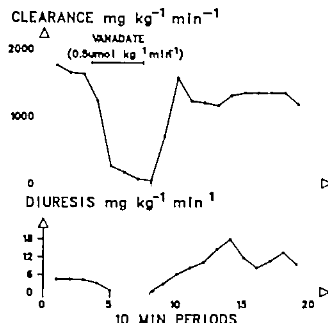


Fig 1 The effect of vanadate on renal clearance of inulin and on urine flow

covery period of at least 60 min was allowed followed by a control period of ½–1 h during which steady state was obtained In the experiments with single injections of vanadate the cat was allowed to recover between the injections.

Analytical procedures ¹⁴C-activity in plasma and urine was measured in a Packard Tricarb Liquid Scintillation Spectrometer as previously described (Krarup et al 1976) Sodium and potassium were determined by flame photometry and chloride by potentiometric titration Plasma vanadate was determined by a method which takes advantage of the shift in equilibrium binding of ⁴⁸V vanadate to Na K ATPase upon addition of an aliquot of serum (Hansen 1980)

The same principle was used for determination of a possible influence of chloralose and its degradation product on the binding of vanadate to Na K ATPase

Calculations Renal clearance of inulin was calculated as urine flow related to body weight multiplied by urine-to-plasma ratio of ¹⁴C inulin Also blood flow was related to body weight (ml kg min) and the unit for pressure was mmHg Arterial conductance was calculated as mean arterial blood flow divided by mean arterial blood pressure portal venous conductance as mean portal flow divided by mean portal pressure and mesenteric conductance as mean portal flow divided by the difference between mean arterial and portal pressure Conductance is thus expressed as ml kg min (mmHg) The results are given as mean values ± S E. and the effect of vanadate was tested by the method of paired comparisons using the Student's *t*-test

RESULTS

Vanadate and kidney function Injection of vanadate in doses up to 1 0 μmol kg⁻¹ did not affect urine production whereas 5 0 μmol kg⁻¹ always decreased urine flow Higher doses in addition to the effect on kidney function often elicited bradycardia cardiac arrhythmia and hypotension In experiments a single injection of 5 0 μmol kg⁻¹ caused a marked transient fall in urine production from 7 94±0 65 to 2 67±0 83 mg kg⁻¹ min and after about 40 min urine production reached a peak value of 9 30±0 74 mg kg min⁻¹ which was significantly higher than control urine flow The urinary excretion rate of sodium in the control period and the post injection period at peak urine production was 0 63±0 13 and 0 69±0 16 μEq kg min⁻¹ (n s) whereas potassium excretion rate increased from 1 87±0 4? to 3 23±0.36 μEq kg min (0 005<P<0 01)

When given as a continuous infusion vanadate did not cause any change in urine production at dose rates up to 0 1 μmol kg min Infusion of 0 5 μmol kg min always decreased urine production and this rate of infusion could be tolerated for up to 60 min Higher dose rates could only be tolerated for a short period of time and caused cardiovascular disturbances as seen with injection of high doses of vanadate A few min after the onset of the infusion of 0.5 μmol kg min urine flow started to fall It remained low during the infusion which lasted about ?0 min and when the infusion was stopped the flow returned gradually within approx 30 min to a value which was slightly but not significantly higher than the control value (see Larsen et al 1979 Fig 1 and Table 1) The mean urine flow in 6 expts was 7 93±1 0? before 0.61±0.2 during and 15 94±3 70 mg kg min⁻¹ after vanadate infusion The urinary excretion rate of sodium and potassium was 0 79±0 17 and 1.82± 0 74 μEq kg min before vanadate infusion and increased slightly after the infusion was stopped to 1 91±0 76 and 3.51±1 28 μEq kg min respectively The post infusion values were not significantly different from the control values The excretion rate of sodium and potassium was not measured during the infusion because of the small amount of urine produced

In four of these experiments mean blood pressure and renal blood flow were also followed During vanadate infusion mean arterial blood pressure increased from 116±5 to 155±5 mmHg (0 020<P< 0 025) renal blood flow decreased from 6.7±1.3 to ? 4±0 3 ml kg min (0 0?0<P<0 025) and renal arterial conductance decreased from 0 058±0 011

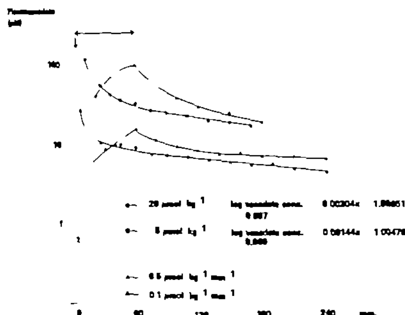


Fig. 3. Plasma concentrations of vanadate-like activity before and following injection or infusion of vanadate.

the renal vascular beds to an increase in conductance in the femoral and carotid vascular beds. The effect of vanadate on blood flow and conductance are illustrated in Fig. 2. The duration of the vascular effect is dose dependent, being about 5 min for the smallest dose and up to 45–60 min for 5.0 µmol kg.

In an attempt to elucidate the vascular action of vanadate the effect of different vasoactive substances on the vascular response to vanadate was tested in 2–3 cats. However, Phenoxybenzamine (1 mg kg⁻¹), Verapamil (10–20 µg kg min), bilateral (1 mg kg) or Nifedipine (50 µg kg) did not change the vascular response to vanadate.

Plasma concentrations of vanadate. In four experiments the plasma concentrations of vanadate after a single injection or continuous infusion of vanadate were followed. The vanadate-like activity in plasma before vanadate was given was in 6 expts. 0.54±0.03 µM. The time concentration curves from the experiments are illustrated in Fig. 3. About 60 min after a bolus injection the curve appears to be monoexponential. From this part of the curve half time (T½) was calculated to 200 min or 150 min when 5 or 20 µmol kg, respectively was given. The solvent space for vanadate in the two experiments was found to be 57 and 50% of body weight, respectively. When comparing the time concentration curves with the observed vascular effects of vanadate, the effects were evoked at plasma vanadate concentrations of about 30 µM.

The effect of vanadate on plasma concentration of N and K. In 6 expts. with continuous infusion (0.5 µmol kg⁻¹ min) vanadate did not change the plasma concentration of Na which was 149±0.3 mEq L before infusion and 149±0.5 mEq L at the end of the infusion. In contrast plasma K increased in 8 expts from 5.27±0.30 mEq L before infusion to 6.40±0.48 mEq L ($0.005<P<0.01$, n=6) during vanadate infusion and returned to 5.39±0.64 mEq L within 1 to 2 h after the infusion was stopped.

Chloralose and vanadate binding. In order to test whether chloralose anesthesia might interfere with binding of vanadate to Na, K ATPase the effect of chloralose and its degradation products, chloralhydrate and trichlorethanol on the binding of vanadate to the enzyme was tested in "in vitro" experiments. Enzyme prepared from ox brain was incubated with ~5·10⁻⁸ M ⁴⁸V vanadate in the presence and absence of the drugs. Addition of 0.05–0.50 mg ml⁻¹ did not significantly change the

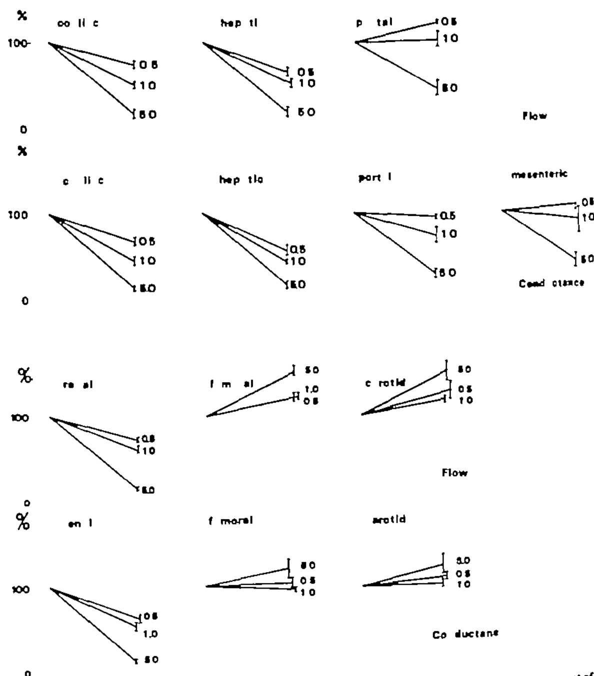


Fig 2 The effect of vanadate 0 5 1 0 and 5 μmol kg on blood flow and conductance expressed in per cent of the control value In the control state the following values were obtained (mean ± S E). Flows (ml kg min). Portal 49 3 6 Hepatic 16 1±2.5 Coeliac 21.2±5.5 Renal 6.0±1 8 Carotid 6.7±0.7 Femoral 4 0±1.3 Conductances (ml kg min (mmHg)). Portal 4 10±0 708 Mesenteric 0. 1 ±0 04., Hepatic 0 133±0 0?6, Coeliac 0.143±0.040, Renal 0 049±0.014 Carotid 0 052±0 007 Femoral 0.034±0 010

9 mmHg ($n=12$) and when 5 0 μmol kg was injected blood pressure increased from 130±8 to 165 ±9 mmHg ($n=1?$) These changes were all highly significant ($P<0\ 001$) The rise in blood pressure was not accompanied by significant changes in heart rate

Portal venous pressure increased from 6 3±0 8 to 8 0±1.2 mmHg ($n=4$) when 0 5 μmol kg was given and from 7 3±1 5 to 10 7±1 8 mmHg ($n=3$) with 1 0 μmol kg These changes were not significant, whereas 5 0 μmol kg caused a significant increase in pressure from 8 0±1 ? to 12.6±1 9 mmHg ($0\ 001<P<0\ 005$ $n=3$) Vanadate did not increase the pressure in the inferior vena cava

Conductance also varied with the amount of vanadate given from a marked fall as observed in

ly ma resulted in a reversible reduction in ... flow and a decrease in renal venous outflow ... and renal venous pressure.

The present experiments have demonstrated that vanadate also constricts other splanchnic vessels. The effect is dose dependent within rather narrow limits, reversible and apparently mediated by a direct effect on the vessel musculature. Simultaneous administration of α- or β-blockers as well as blockers of Ca^{++}-transport did not hinder or modulate the vanadate induced constriction. In contrast to the effect on splanchnic vessels vanadate caused an increase in blood flow in the femoral and carotid artery while the vascular conductance increased or remained unchanged.

Similar vascular changes caused by vanadate were suggested years ago by Jackson from oncometer measurements (1912). Recently Steffen et al (1979) and Inciarte et al. (1979) reported that i.a. administration of vanadate produced constriction of all components of the skin and skeletal muscle vascular bed of the dog forelimb as well as a rise in coronary resistance. In continuation of these experiments Hout et al. (1979) found that vanadate increased tension in the isolated saphenous vein from the dog at a concentration of 10^{-4} M and this effect was not affected by Phentolamine. In the perfused rat liver vanadate at concentrations from 9–50 μM caused dose dependent fall in hepatic vascular conductance (Thomsen & Larsen 1980). Finally it has been shown by Bell et al. (1979) that vanadate caused vasoconstriction in the perfused, irrigated gills of the eel, this effect was completely inhibited by noradrenaline.

It is known that vanadate influences several enzymes (see Simons 1979) but the action of vanadate on vascular smooth muscle resembles that of ouabain (Treat et al 1971 Friedmann et al 1973 Webb & Bohr 1978). It might therefore be expected that the vascular effect is caused by an inhibition of Na, K-ATPase in the vascular smooth muscle. This is supposed to lead to a secondary increase in the intracellular concentration of Ca^{++} with contraction of the muscle cells as a consequence (Schwartz et al 1973 Blaustein 1977). The results of Hout et al (1979) are in conflict with this. They found in experiments on the isolated saphenous vein that vanadate did not influence the ouabain-sensitive ^{86}Rubidium uptake in vascular smooth muscle which is supposed to be a measure of Na, K-ATPase activity. Furthermore the observed lack of effect of Ca -transport blockers on vanadate induced vasoconstriction seems to speak against this hypothesis.

The authors are indebted to Dr O Hansen, Institute of Physiology University of Aarhus for determination of vanadate. W also thank M K. Donby and A. Molbeck for skilled technical assistance

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equilibrium binding level of vanadate established during 60 min of incubation

DISCUSSION

The inhibitory effect of vanadate on Na K ATPase (Charney et al 1975 Josephson & Cantley 1977 Cantley et al 1977) has renewed the interest for the biological actions of vanadate Among the recen ly discovered effects are the diuretic and natriuretic effects observed in unanesthetized rats (Balfour et al 1978) in which vanadate (approx 5 μmol kg given i v) increased urine flow and renal sodium excretion rate by 14–16 times These results were only partly confirmed by Bonventre et al (1979)

The present experiments have demonstrated that vanadate given i v in comparable amounts and giving approx the same plasma concentrations as found by Balfour et al (1978) inhibits urine production in the anesthetized cat The fall in urine production was reversible and accompanied by marked and similarly reversible constriction of the renal vasculature and fall in renal blood flow

A simultaneous fall in glomerular filtration rate was seen whereas the urine to-plasma ratio of 4C inulin and thus the fractional water excretion rate was not significantly affected by vanadate This was even the case under conditions where tubular reabsorption and thereby this ratio was markedly reduced by Furosemide

The application of a flowmeter on the renal artery which involves denervation did not affect renal function in the control period or the action of vanadate This indicates a direct effect of vanadate on vascular smooth muscle

The observed fall in urine production accompanied by almost unchanged fractional water excretion rate is characteristic for the high degree of glomerulotubular balance which has been found during acute reductions in glomerular filtration rate caused by partial clamping of the renal artery or by other means (see Pitts 1974) The fall in urine production caused by vanadate may therefore be explained by a fall in glomerular filtration rate secondary to renal vasoconstriction and a corresponding fall in glomerular filtration pressure

This effect is in contrast to the diuretic action in the rat (Bonventre et al 1979) where vanadate was without effect on glomerular filtration rate but caused an increase in fractional water excretion rate —together with an increase in sodium excretion rate Their results are consistent with an inhibition of Na K ATPase and thereby salt and water reabsorption in the proximal renal tubules although it might be expected that a fall in proximal fluid reabsorption would cause a fall in glomerular filtration rate

It thus appears that in the cat the effect of vanadate on renal function is dominated by a constriction of renal blood vessels whereas in the rat an inhibition of Na K ATPase in the renal proximal tubules is the main action However the action of vanadate in the rat seems to be highly dependent on the dose given and the route of administration (Bonventre et al 1979) and this together with an observed increase in blood pressure indicates that vanadate under certain conditions may elicit vasoconstriction eliminating or reducing the diuretic effect of its action on tubular Na, K ATPase. Likewise it cannot be excluded that vanadate inhibits proximal reabsorption of salt and water also in the cat renal tubules but that this effect is obscured by its marked action on renal vessels i e all nephrons might be urine producing but at a markedly reduced glomerular pressure It may also be that vasoconstriction interferes with the contact of vanadate with Na K ATPase and that the local hypoxia produced might favour the reduction of vanadate (+5 oxidation state) to e g vanadyl (+4 oxidation state) which is less effective in inhibiting Na, K ATPase (Cantley & Aisen 1979 Grantham & Glynn 1979)

Although the present results are in contrast to those obtained in rats they are in accordance with experiments on Pentobarbitone-anesthetized dogs as reported by Mills & Newport (1979) They found that infusion of vanadate (0 54 μmol min) into the renal artery caused hypertension renal vasoconstriction and a 50–60% reduction in both urine production and glomerular filtration rate Urinary excretion rate of sodium was not affected but a slight fall in plasma sodium concentration was observed In the present experiments plasma Na -concentration was not affected by vanadate, whereas a significant rise in plasma K -concentration was observed This rise can only be partly explained by decreased renal excretion of potassium during infusion of vanadate and may be caused by inhibition of Na K ATPase elsewhere Also Inciarte et al (1979) state in a preliminary report that vanadate given i v to dogs in doses of 0.–1 0 μmol

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Cerebrovascular sympathetic denervation and blood brain barrier function in conscious rats

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Electrical stimulation of the sympathetic nerves to the cerebrovascular bed enables the resistance vessels to better withstand a high blood pressure in terms of blood-brain barrier integrity Sympathetic denervation could hence be xpected to lead to decrease in cerebrovascular tone and increased vulnerability of the blood-brain barrier I the present study acute hypertension was induced in conscious unrestrained rats by administration of angiotensin or bicuculline. The albumin leakage into the brain, as studied by Evans blue-albumin and ^{125}I labelled human serum albumin, was not enhanced in acutely or chronically sympathectomized rats compared to controls.

Key ords. Cervical ympathectomy acut hypertension blood-brain barrier albumin tracers

The brain vascular bed receives an ample supply of sympathetic adrenergic nerves originating in the superior cervical ganglia (Nielsen & Owman 1967) A reduction of the cerebral blood flow (CBF) by about 10–15% is obtained upon sympathetic nerve stimulation at normotension and normocapnia (for review see Edvinsson and MacKenzie 1977 Heistad et al. 1978 Purves 1978). The sympathetic nervous activity thus lead to an increase in cerebrovascular tone via activation of cerebrovascular alpha-adrenergic receptors (Edvinsson & Owman 1974). There is evidence supporting physiological role of these nerves as reflected in the recent observation that stimulation of the sympathetic trunk in the neck extends the upper limit of autoregulation towards higher blood pressure levels (Bill & Linder 1976, Edvinsson et al. 1976, MacKenzie et al 1977 MacKenzie et al 1979)

In acute hypertension the mechanical stress on the cerebral vessels increases the cerebrovascular permeability to protein (for references see Johansson 1976) Concomitant vasodilatation—as induced by e g. papaverine bicuculline, amphetamine or hypercapnia—enhances the extent of blood-brain barrier (BBB) dysfunction (Johansson 1974 Johansson & Nilsson 1977 Carlsson & Johansson 1978 Hardebo 1980) Stimulation of the sympathetic nerves to the brain vessels enables the resistance vessels to better withstand a sudden blood pressure increase I e protein e travasation and flow incre ment are prevented in acute hypertension (Bill & Linder 1976) An ameliorative effect of sympathetic stimulation is also obtained when acute hypertension is combined with metabolic vasodilatation (Johansson & Lund 1978) Sympathectomy on the other hand, might be xpected to result in a reduction in cerebrovascular tone and, possibly an increased vulnerability—in terms of BBB function—to acute hypertension. This condition is examined in the present study with particular attention to the preexisting vascular tone in the test situation.

MATERIAL AND METHODS

The xperiments were performed on 49 conscious male Sprague-Dawley rats (200–300 g b. wt.). A catheter was implanted in the aorta under barbiturate anesthesia 2 3 days prior to the experimentation with induction of acute hypertension. Simultaneously the right external jugular

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Table ⁹⁹HSA content in the cerebral cortex caudate nucleu and erebellum after acute hypertension induced by ⁹0 µg kg⁻¹ angiotensin II amide I in awake unrestrained rats at various time intervals after cervical sympathectomy (sy-ect) and in ontrols

For details of the groups, see Table 1 The relative activity is show as the ratio of activity between brain and blood × 100 Mean values ± S E.

Experimental groups	n	Cortex dx	Caudat nucl dx	Cortex sin	Caudate nucl sin	Cerebellum
Control	7	0 07±0 01	0 06±0 01	0.07±0 01	0.06_0 01	0 15±0 03
Acute sy-ect dx	6	0 07+0 01	0 04±0 01	0 06_0 01	0 03 0 01	0 08+0 01
Acute sy-ect dx	8	0 08±0 01	0 07±0.01	0.08±0 01	0 09±0 01	0 12_0.01
Chronic sy-ect dx (3) bilat (6)	9	0 06±0.01	0.04±0.01	0 06±0.01	0.05±0 01	0.10±0.02

n that these nerve fibres participate in the regulation of the cerebrovascular tone, however only to a considerable extent at extreme situations such as marked acute systemic hypertension. In this situation a supportive function of the sympathetic innervation has been demonstrated in anesthetized animals in terms of ability to maintain both autoregulatory capacity and blood-brain barrier integrity (see Introduction) A decreased ability to withstand an abrupt increase in intraluminal pressure has been demonstrated following sympathectomy in animals lightly anesthetized with nitrous oxide and immobilized by tubocurarine (Edvinsson et al 1977) The high initial mean arterial pressure in these animals (MAP 140–160 mmHg) suggests a stress situation as do high circulating levels of adrenaline and noradrenaline in anesthetized or awake, immobilized animals as compared to freely moving animals (Depocas & Behrens 1977 Bühler et al 1978, Saavedra et al. 1979).

The present results indicate that a tonic influence on the blood-brain barrier function of the sympathetic nerve is minor or absent in conscious unrestrained rats subjected to acute hypertension induced by angiotensin II and bicuculline The initial sympathetic activity is probably low as compared to anesthetized rats and the increase in blood pressure caused by an i injection of a vasoactive drug is not likely to be accompanied by an emotional arousal of a kind that may occur in connection with blood pressure elevations during daily life It may therefore not seem urprising that sympathectomy did not influence the angiotensin II-induced opening of the barrier in our study However sympathectomy did even not increase the protein leakage in rats after bicuculline administration The significantly lower extravasation in sympathectomized rats given bicuculline is likely to be related to the lower maximum blood pressure in these rats (Tables 1 and 3) Whereas the magnitude of the blood pressure increase as well as the maximum pressure level is of importance in acute hypertension per se the maximum pressure is the main factor responsible for the protein leakage in those experimental models in which hypertension is combined with pronounced cerebral vasodilatation (cf Johansson & Nilsson 1977)

Epileptic discharge is accompanied by an intense sympathetic acti ation which is the cause of the blood pressure increase In a recent study (Moeller

Table 3 ⁹⁹HSA cont nt in th erebral cort thalamus and cerebellum after injection of 1.2 mg kg bicuculline in aw ke nrestra ned rat at arious time int rv ls after acut (20–75 min) or hronic (10 12 d) sympathectom (sy-ect) nd in controls

The relative activity is shown as the ratio of activity between brain and blood 100. Mean values ± S.E.

Experimental group	n	Cortex dx	Thalamus dx	Cortex sin	Thalamus sin	Cerebellum
Control	10	0.19±0 02	0 41±0 04	0 20±0 02	0 44±0.04	0 18±0.03
Acute sy-ect d (3), bilat (1)	4	0 07±0 01	0.17±0 09	0 07±0.02*	0.19±0 08	0 09±0 02***
Chronic sy-ect bilat	5	0 08±0 01	0 09±0 02**	0 09±0.02*	0 11±0.03	0 10±0 02*

* $P<0.05$ ** $P<0.01$ for difference from control group

Table 1 *Mean arterial pressure (MAP) before and after a blood pressure increase induced by angiotensin (20 µg kg⁻¹) or by bicuculline (1.2 mg kg⁻¹) in rats after acute (4 min–5 h) or chronic (4–10 days) cervic sympathectomy (sy-ect) and in controls*

The sympathectomy was performed either by removal of the superior cervical ganglion (gangl) or by section of the preganglionic nerve (pregangl) P_aCO_2 and P_aO_2 immediately before the induction of hypertension Mean value±S E

Experimental group	n	Initial MAP (mmHg)	Maximum MAP (mmHg)	ΔMAP (mmHg)	P_aCO_2 (kPa)	P_aO_2 (kPa)
Angiotensin						
Control	7	113±5	117±5	64	4 1±0 1	1 0±0 3
Acute sy-ect dx (gangl)	6	95±5	188±5	93	3.8±0 1	12 2±0 2
Acute sy-ect dx (pregangl)	8	110±3	176±5	66	3 9±0 1	13 7±0 5
Chronic sy-ect dx (3) bilat (6) (pregangl)	9	111±3	188±3	77	4 0±0.1	12.5±0 2
Bicuculline						
Control	10	117±5	189±3	72	4.5±0 1	1 ±0
Acute sy-ect dx (3) bilat (1) (gangl)	4	101±4	174±3	73	4 0±0 1	12.5±0 5
Chronic sy-ect bilat (pregangl)	5	118±6	172±5	54	4 1±0.	12.1±0

vein was cannulated for injection of dyes and tracers except in the animals being subjected to acute sympathectomy at a later step In these latter rats the external jugular vein was cannulated immediately prior to the sympathectomy The free ends of the aortic and jugular catheters were exteriorized on the back of the neck

Sympathectomy Acute unilateral in a few animals bilateral excision of the superior cervical ganglion was performed under ether anesthesia In some experiments the preganglionic nerve trunk (about 5 mm below the ganglion) was cut at various time intervals (4 min to 5 h) before the induction of acute hypertension Chronic unilateral or bilateral sympathectomy was performed 4–12 days before the experimentation

Induction of acute hypertension The aortic catheter was connected to a transducer and the resting mean arterial pressure (MAP) was recorded in conscious unrestrained rats Blood gases (P_aO_2 and P_aCO_2) were repeatedly determined The following drugs were used for detecting blood-brain barrier opening: ^{131}IHSA (human serum albumin 100 µCi kg⁻¹) and Evans blue (1 ml of a 2% solution in sal ne) which in vivo binds to serum albumin A rise in MAP was induced by an i v bolus injection of either angiotensin II amide (Hypertensin CIBA 20 µg kg⁻¹) or bicuculline (1.2 mg kg⁻¹)—a drug that provokes epileptic activity and hemodynamically gives an abrupt rise in blood pressure combined with pronounced cerebral vasodilation The resulting BBB dysfunction is exclusively pressure-dependent (Johansson & Nilsson 1977)

3 min after induction of a pressure increase the rats were anesthetized with pentobarbital i v and the brains were perfused via the left heart ventricle w th saline in situ for 30 s to remove the blood from the cerebral vessels After macroscopic examination of Evans blue-albumin extravasation the radioactivity of predetermined brain regions (see Tables 2 and 3) was measured in a gamma counter The extravasation of ^{131}IHSA was expressed a the percentage ratio of brain and blood activity i e 100× [CPM/mg brain tissue over CPM/mg blood] Blood samples were taken immediately before starting the perfusion through the heart Statistical differences were evaluated with Wilcoxon s rank sum test

RESULTS

A patchy or dot like extravasation of Evans blue albumin was noted in all rats little in rats given angiotensin II and more pronounced particularly in the thalamus of animals given bicuculline Sympathectomy (unilateral or bilateral) did not significantly alter the amount of extravasation of the tracers ^{131}IHSA or EBA in rats given angiotensin I (Table 2) This was irrespective of whether ganglionectomy was performed acutely (to avoid denervation hypersensitivity) or chronically or whether preganglionic denervation was performed to avoid initial release from the postganglionic neuron caused by manipulation of the ganglion

DISCUSSION

The density of the cerebrovascular sympathetic plexus is comparable to that of various other peripheral vascular beds and the nerve fibres extend at least in baboon and cat along the cerebrovascular tree down to the level of small intraparenchymal arterioles (Edvinsson & Owman 1977 Cervós Navarro & Matakas 1974) The functional importance of this innervation has been extensively studied during the last few years (for review see Edvinsson & MacKenzie 1977) The major opinion emanating from studies on sympathetic stimulation

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et al 1979) the cervical sympathetic nerve activity was registered before and after injection of bicucul line in cat and shown to increase 3-fold concomitant ly with the increase in blood pressure In agreement with the present results unilateral sympathectomy did not increase the extravasation of protein in the denervated side of the brain It is possible that the metabolic vasodilatation induced by bicuculline may have masked a constrictory effect of the sympathetic nerves The fact that sympathetic nerve stimulation significantly decreases bicucul line induced protein leakage in anesthetized rats (Johansson & Lund 1978) does not rule out this possibility as electrical stimulation may not be directly comparable with physiological stimulation

It may be argued that an abrupt rise in blood pressure as induced presently might preceed a reflectory sympathetic vasoconstriction and therefore the present tests would not allow for interpretations of a role for the sympathetic innervation in BBB function A less rapid rise in pressure did not however potentiate the BBB opening following sympathectomy (unpublished observation) further a lowering in the rate of pressure increase would probably also allow myogenic autoregulation to compensate for the pressure increase

In spite of all recent evidence that sympathetic nerves do play a role in the regulation of cerebral blood flow and blood-brain barrier integrity at high levels of blood pressure the physiological importance of this mechanism remains to be elucidated

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Dissociation of electrical and mechanical activity caused by vibrations in the spontaneously active smooth muscle of the rat portal vein

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SJÖQVIST A. & LJUNG B Dissociation of electrical and mechanical activity caused by vibrations in the spontaneously active smooth muscle of the rat portal vein. Acta Physiol Scand 1980, 110 381 384 Received 5 March 1980. Department of Physiology University of Göteborg, Box 33031 S-400 33 Goteborg Sweden.

The effects of vibrations on the electrical membrane discharge and on the contractile force of the spontaneously active smooth muscle of isolated rat portal vein were studied. The electrical activity was recorded extracellularly and quantitatively related to the mean active force. Sinusoidal vibrations (40 Hz, 2.5–3.0% tissue length peak to peak) applied in the longitudinal direction of the smooth muscle, caused prompt and reversible reduction of active force but neither the patter of the phasic contractions nor the electrical membrane discharge was altered The degree of inhibition of mechanical activity increased with vibration amplitude activity being 50% of control at vibration amplitude of 12±4% (mean ± S.D =8) It is concluded that the induced length changes caused prompt dissociation between electrical membrane discharge and mechanical force development in the vascular smooth muscle This finding adds support to the previously forwarded hypothesis that vibrations cause inhibition of contracting muscle by direct action on the contractile process.

Key words Vascular smooth muscle portal vein, electrical activity contractile force, vibrations

Vibrations have been found to reduce active force development in isolated vascular smooth muscle (Ljung & Sivertsson 1975 1980) There is indirect evidence that this inhibition of active force in smooth muscle is a direct action by the imposed length changes on the contractile machinery possibly due to an increased rate of detachment of actin-myosin cross-links (for reference see Ljung & Sivertsson 1980) as originally suggested for skeletal muscle (Joyce, Rack & Westbury 1969)

In the present experiments, the effects of longitudinal vibrations on the spontaneously active smooth muscle of the rat portal vein have been quantitated in terms of electrical discharge of the membrane and compared to the mechanical force output. The method implies a correlation between the integrated electrical and mechanical activities respectively when recorded by a method analogous to that used by Ljung & Stage (1970). The results demonstrate that a dissociation between electrical and mechanical activity occurs under the influence of vibrations.

MATERIAL AND METHODS

Isolated portal veins from male rats of the Sprague Dawley strain, weighing 200–250 g were studied Under brief ether anaesthesia the rats were sacrificed and the portal vein was carefully dissected free of surrounding tissue tied at both ends with fine silk and isolated. It was mounted vertically in an organ bath filled with modified Krebs solution at 38°C. The lower end was firmly attached to submerged isometric transducer with compliance of 0.2 mm/N The transducer utilized semiconductor element (AE 803P Akers Electronics, Norway). The upper end was tied to steel rod extending from the core of an electromagnetic vibrator (Mod 201 Ling Dynamic Systems Ltd, Great Britain).

The vein was stretched to resting passive force of 4 mN and left to accommodate for at least 1 h before the experiment was started. The tissue lengths at 4 mN passive force ranged between 3 0–5 4 mm when measured by means of dissection microscope equipped with micrometer eye piece.

The electromagnetic vibrator moved the steel rod in the vertical direction, i.e. it imposed length changes in the direction of the smooth muscle cells of the heavy longitudinal outer media layer of the portal vein (cf Johansson et al. 1970) An in-built photo-electric device

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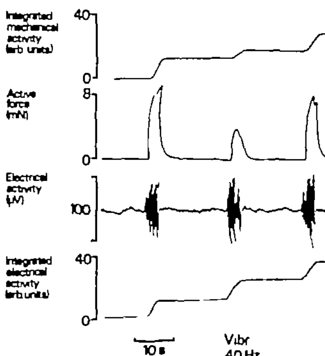


Fig 1 Tracings of electrical and mechanical spontaneous activity of the rat portal vein Three consecutive bursts of electrical discharge and associated contractions are seen. Vibrations (40 Hz 8% tissue length) were applied for 10 s (Vibr) during which period the middle contraction wave occurred Note that the contraction amplitude but not the electrical discharge was reduced Neither was time interval between contractions altered

registered the position of the core of the vibrator and a potential proportional to the displacement was generated (1. V/mm displacement) and fed into a control amplifier (L. Stage unpublished) The control amplifier was connected to the core of the vibrator and to an input signal and forced the displacement signal to follow the input signal very closely Both signals were continuously monitored on a dual beam oscilloscope This control system made it possible to induce exact length changes which followed the predetermined characteristics of the input signal with regard to wave form frequency and amplitude In the present set of experiments the input signal was sinusoidal with a frequency of 40 Hz and varying amplitudes. Adjustments were made so that the mean passive tension remained constant during the vibrations.

The electrical activity was recorded between an exploring extracellular electrode and a reference electrode The silver recording electrode was connected to the tissue via a glass tube with a small tip (inner diameter about 0 1 mm) It was filled with Krebs solution and an Ag-AgCl wire was inserted into the tube. The tip of the capillary bridge was placed against the adventitial surface of the portal vein. The reference electrode was an Ag AgCl wire connected to the bath via an agar-saline bridge The electrodes were connected via capacitors to a polygraph (Grass preamplifier mod 7P1E) The force transducer was connected to the polygraph for isometric recording of active (spontaneous) force developed by the longitudinal smooth muscle layer of the portal vein The active force produced was integrated over 1 min intervals. The electrical recording was rectified and integrated simultaneously on a separate channel of the polygraph

The modified Krebs solution used had the following composition in mM NaCl 12.. KCl 4 73, $CaCl_2$ 2.0 $MgCl_2$ 1 19 $NaHCO_3$ 15.5 KH_2PO_4 1 19 glucose 11 5 It was continuously bubbled with 4% CO_2 in O_2.

Statistic Two-tailed Student's t test was used for calculation of the significance of displacement from control values.

RESULTS

In the control situation the longitudinal smooth muscle of the rat portal veins displayed the typical activity pattern of phasic spontaneous contractions at a rate of 1-? contractions per min with intervening periods of complete relaxation to the baseline of passive force Associated with each contraction a burst of action potentials was recorded the onset of which slightly preceded the development of active force Fig 1 illustrates an experimental sequence, where tracings of the electrical and the mechanical activities respectively of a spontaneous contraction in the control situation are shown The second contraction was recorded during exposure to sinusoidal vibrations (40 Hz 8% peak to peak amplitude of tissue length) and a subsequent third contraction recorded after cessation of vibrations. In addition to the recordings of active force and electrical discharge the integrals of each of these activities (see methods) are shown in separate tracings of Fig 1 It is apparent that the vibrations selectively reduced the amplitude of the contraction whereas the duration of the contraction and the interval between the three contractions remained unchanged Furthermore the electrical discharge was not altered In order to study the relative effect of vibrations on electrical and mechanical activity respectively the integrated values obtained over one min periods were averaged during a 15 min control period and during subsequent 5 min periods of vibrations at increasing vibration amplitudes. At the end of the vibration exposures the control activity was again quantitated In Fig ? the averaged integrated values of the electrical and mechanical activity during exposure to vibrations at 40 Hz and of the amplitudes indicated (% tissue length peak to peak) have been expressed as a percentage of the mean integrated electrical and mechanical activity during

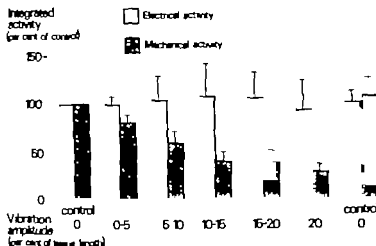


Fig Effects of vibrations (40 Hz) at graded amplitudes on integrated electrical discharge (open bars) and on integrated active force (hatched bars) of the spontaneously active longitudinal muscle of the rat portal vein. In each experiment the mean activities recorded during 5 one minute periods of vibration exposure at given vibration amplitude were xpressed as percentage of the averaged 15 one minute period in the (initial) control situation. Mean ± S D from 8 experiments.

xposure to ibrations at 40 Hz and of the amplitudes indicated (% tissue length, peak to peak) have been expressed as a percentage of the mean integrated electrical and mechanical activity over the initial 15 min control period The integrated mean active force was diminished to an extent which depended on vibration amplitude ($P<0.05$ for all groups) whereas the integrated electrical activity was not significantly altered ($P>0.05$ for all groups) The vibration amplitude which at 40 Hz would cause 50 % reduction in mean active force (as determined by interpolation in the individual experiment, see methods) amounted to 1 ±4 4 % peak to peak of total tissue length (mean ± S D)

During the second control period following cessation of vibrations, neither electrical nor mechanical activity was significantly different from the initial control values (Fig. 2)

DISCUSSION

The ibrations applied in the longitudinal direction of the spontaneously contracting isolated smooth muscle of the rat portal vein educed the developed active force while the quantitated electrical discharge and the phasic contractile pattern remained unaltered The force output decreased with increasing amplitude of the applied oscillating length changes in the range 2.5 to 30 % of the tissue length. This confirms previous findings in vascular smooth muscle (Ljung & Sivertsson 1975) as well as in isolated myocardium (Vukas Sivertsson & Ljung 1978).

In the present experiments the electrical membrane discharge was recorded extracellularly by means of saline filled large glass electrode This volume conductor recording system measured bursts of compound impulse activity in the neighbourhood of the electrode tip (cf Ljung & Stage 1970) During well synchronized smooth muscle activity which normally prevails in homogeneous sections of the longitudinal muscle layer of the rat portal vein the bursts of action potentials will spread throughout the entire muscle Thus, recorded electrical activity can be considered to reflect the total electrical discharge in the longitudinal smooth muscle layer of the portal vein (cf Ljung & Stage 1970). The rectified AC signal has been quantitated by means of integration over 1 min periods I the control situation there seemed to be a linear relationship between the electrical activity and the mechanical activity quantitated as the integral of the force signal obtained over the same 1 min period. When vibrations were applied to the tissue no apparent artifacts were induced in the electrical recording (Fig. 1) and the smooth muscle maintained the same rhythm of contractions. Thus, our finding of unchanged electrical activity but

graded reductions of mechanical activity during vibrations at varied *amplitudes demonstrates that* the longitudinal oscillations cause a selective interference with the mechanical force generation of the smooth muscle Joyce Rack & Westbury (1969) proposed that in skeletal muscle vibrations increased the rate of detachment of actin–myosin cross-links and the present results strongly indicate *that this is also true of smooth muscle* (see Ljung & Sivertsson 1980)

On the other hand it is well known that in some situations length alterations induce marked changes in electrical discharge and associated changes in mechanical activity Johansson & Mellander (1975) *have shown that slow elongation of the portal vein* (0 7% *muscle length/s for 2 min) caused increased* spike discharge and contraction rate whereas slow passive shortening caused decreased electrical and mechanical activity Cyclic slow stretches and shortenings have been found to make the phasic contractions cluster during the periods of increasing length and to become rarefied during the shortening periods As the frequency of such cyclic lengthening and shortening increases the membrane effects no longer become manifest in the rat portal vein since the inhibitory effect will apparently counteract the excitatory effect (Sjöqvist & Ljung to be *published) However Ohhashi et al (1979) have* reported that microvibrations" (1–80 Hz, amplitudes less than 2% tissue length) may evoke phasic contractions at an increased rate after a latency of about 1 min in smooth muscle of the ureter and portal vein of the dog In our experience such an effect does not occur in the isolated rat portal vein unless experimental period extends to 6–8 h The membrane stability of the smooth muscle seems to decrease progressively in the course of an in vitro experiment and thus the membrane sensitivity to stretch is increased Possibly such *changes in the membrane properties may occur* more rapidly *in the larger isolated smooth muscle* specimens studied by Ohhashi et al (1979) than in the thin-walled rat portal vein preparation

The biological relevance of vibrations to vascular smooth muscle *in the physiological* situation is not clear In a recent review Dobrin (1978) reports that with each cardiac cycle conduit arteries undergo 8–10% oscillation in external diameter or about 15% oscillation in internal diameter The basal frequency of such oscillation *would be the* same as the heart rate which seems to be in the lower range of frequencies which may exert significant inhibitory *effects However* the pressure curve is not sinusoidal and many harmonics with higher frequencies but lower amplitudes are present It remains to be clarified whether their amplitudes are high enough to reduce active force generation

Under occupational conditions such as work with vibrating tools and in pathological conditions such as arterial stenosis vibrations of considerable frequency and amplitude may occur locally Such vibrations seem likely to interfere with the contraction process in the vascular wall as previously suggested (Sivertsson & Ljung 1976)

Thanks are due to Ms Ann Kjellstedt for technical assistance to Mr Lars Stage for construction of electronic equipment and to Ms Lena Ziden for secretarial aid

Supported by the Swedish Medical Research Council (Grant No 3884) and by AB Hässle

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Characterization of contractions in mechanically disaggregated myocardial cells from the rat

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LEHTO H. & TIRRI R. Characterization of contractions in mechanically disaggregated myocardial cells from the rat. Acta Physiol Scand 1980. 110: 385–389. Received 10 March, 1980. ISSN 0001-6772. Laboratory of Animal Physiology, Department of Biology, University of Turku, Finland.

Myocardial cell groups, mechanically disaggregated from the rat heart, beat spontaneously in medium which resembles intracellular fluid. The cells, initially functioning in an uncoordinated way, i.e. each cell in its own rhythm, gradually become coordinated as a function of time. After 90 min of incubation at 15°C all the cell groups were functioning in a coordinated way. The initiation of coordination was temperature-dependent. Lowering the temperature from 15°C to 10°C significantly delays the coordination, only 75% of the cell groups being coordinated after 90 min. The contraction wave in coordinated cell groups was very slow: 90±7 µm/s at 20°C, 100±6 µm/s at 30°C and 100±7 µm/s at 35°C. The velocity of contraction waves was not significantly temperature-dependent, suggesting a nonenzymatical type of propagation. It also seems too slow to be an electrical event. Apparently the contraction waves are propagated by diffusion of Ca^{++}. The addition of caffeine to the medium hastened the coordination of contractions but Na-azid strongly inhibited it. The results indicate that the sarcoplasmic reticulum functions in the coordination of contractions, probably by releasing Ca^{++} for the activation of the myofilaments.

Key words: Rat, myocardial cells, in vitro function

The contraction of a myocardial cell is initiated by an action potential, the depolarization of sarcolemma. The binding of Ca^{2+} with the troponin C terminates the series of events preceding the contraction. The excitation-contraction coupling process is still partially unclear, especially concerning where and by what mechanism Ca^{++} is released into this system. Two possible mechanisms have been suggested: a Ca^{++} induced release of Ca^{++} from the sarcoplasmic reticulum (Fabiato & Fabiato 1975, 1977) and depolarization-induced release of Ca^{++} from the sarcoplasmic reticulum (Endo 1977, Thomas & Endo 1975). On the other hand there are also suggestions that the major source of coupling Ca^{2+} is external to the sarcolemma and that this superficial Ca moves nonelectrogenically during excitation-contraction coupling (Langer 1976).

A technique has been developed for isolating mammalian myocardial cells so that single cells are able to contract rhythmically (Bloom 1970, 1971). The cells function in an incubation medium which resembles intracellular fluid. The cells are suggested to beat by means of extracellular Ca^{++}, the sarcolemma being in these circumstances highly permeable to divalent cations (Bloom & Mulholland 1976). Ca-influx covaries with contraction frequency in contrast to Ca-efflux, which does not (Bloom 1978).

Using Bloom's technique we (Pelkonen & Tirri 1977) found that mechanically disaggregated cell groups from the rat ventricular myocardium can beat in an uncoordinated way, i.e. each cell at its own rhythm, or in a coordinated way where one cell serves as a pacemaker. The aim of this study is to further characterize the function of these isolated cell groups and especially to attempt to find out the role of Ca^{++} in the contraction and relaxation processes.

MATERIALS AND METHODS

Preparation of cell groups

Sprague-Dawley rats of both sexes were used at the age of 7–14 days. This age-group was chosen because we had

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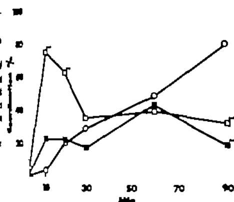


Fig 2 The effect of caffeine on the coordination of the cell groups incubated in HM at 10°C. At every incubation time (abscissa) the percentage of coordinated cell groups (ordinate) calculated In the caffeine experiments, 16–19 cell groups were used at every incubation time and 23–35 control experiments at every incubation time. The caffeine concentrations were 10^{-4} M (□—□) and 2×10^{-4} M (■—■). The points marked with asterisks differ significantly from the controls ($P<0.01$).

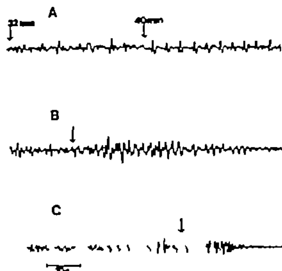


Fig 3 (A) The coordination of contractions registered with the chart-recorder The cell group beating in HM gradually becomes coordinated, which can be seen as larger and regular-shaped wave on the right. (B and C) The effect of caffeine on the uncoordinated contractions. The addition of caffeine is marked with an arrow The caffeine concentrations were 1 mM (B) and 2 mM (C).

incubation temperature was 10°C The difference in coordination in these two temperatures was statistically significant ($P<0.05$).

Effect of caffeine

Caffeine (10^{-4}M), added to the incubation medium at 10°C, accelerated the appearance of coordination (Fig 2). After 10 and 20 min of incubation the differences from the control groups in coordination were statistically significant ($P<0.001$) At 30 and 60 min of incubation however there were no differences from the control groups, and at 90 min caffeine had even significantly delayed the coordination ($P<0.005$) Twice the concentration of caffeine initially slightly stimulated the coordination but later had an inhibitory effect on it. In many cases, however hen incubated for more than 10 min caffeine caused a permanent partial contracture in cells and this made the observation concerning the coordination more difficult. With still stronger caffeine concentration (5×10^{-4} M) this type of permanent contracture started so soon (in 5–10 min), that the allocation of function into the coordinated or uncoordinated type as impossible

Fig 3 shows some examples of the xperiments here contractions in the cell group were registered with the chart-recorder connected to the photoamplifier Fig 3 A is an original recording of contractions from a cell group incubated for 30 min at 15°C The coordinated contractions are seen as larger regular-shaped waves on the right, which coincided with the coordinated contractions as watched on the microscope Fig 3 B shows a re cording of contractions when caffeine (1 mM) was added into the examination chamber after 4 min of incubation as indicated by an arrow Caffeine changes the uncoordinated contractions to the coordinated type but after some minutes of incubation with caffeine the volume of contractions starts to decrease A similar effect can be seen using a larger concentration (? mM) of caffeine (Fig. 3 C) when the depressing effect with a complete contrac ture follows the coordination in about 10 s

Effect of azide Fig. 4 shows the effect of Na-azide mitochondrion poison, on the coordination of contractions It seems that 5 mM NaN strongly inhibited the coordination in the cell groups After incubating with azide for 60 and 90 min the number of coordinated cell groups was significantly less than in the pure HM ($p<0.01$) In an additional test series sing 50 mM NaN not a single coordinated contraction was found with the uncoordinated contractions, although weakened, still continuing.

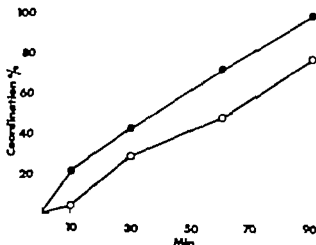


Fig 1 The coordination of contractions in the cell groups over time at 10°C (O—O) and at 15°C (●—●). At every incubation time (abscissa) the percentage of coordinated cell groups (ordinate) is calculated from 19–35 cell groups After 90 min of incubation the coordination at different temperatures differs significantly ($P<0.05$)

earlier observed that myocardial cell from young animals functioned more regularly in these experimental conditions (Pelkonen & Tirri 1972) The rats were killed by decapitation and the hearts were removed and washed in ice-cold homogenization medium (HM) made according to Bloom (1970) It contained (mmoles/l) KCL 104 $MgCl_2$ 5 Na_4ATP 5 potassium oxalate 2 glucose 7 and potassium-phosphate buffer 18 at pH 7 0 The ventricles were dissected free from atria and homogenized in a cutter homogenizator (50000 rpm) for 6–10 s in 10 ml of ice-cold HM The homogenization did not simply break the cells but rather cut and tore the ventricles into cell groups of different sizes Most of them were flat and elongated The fragments selected for the experiments were 100–150 μm in length being 6–8 cells broad 3–4 cells long and one or two cells thick

Coordination experiment

A *Method using a video-tape recorder* The homogenate was kept on ice for 15 min to get the cell groups to bottom A sample of 0 1 ml from the bottom was pipetted into a test tube within 0 7 ml of fresh HM or HM with the drug to be tested Then a sample of 5–10 cell groups was transferred from this tube onto a depression slide and covered with a glass cover The incubation times the temperatures and the drug concentrations are given in the figures The samples was promptly checked with a microscope with phase contrast optics and ocular micro-scale and the movements of each contracting cell group found were recorded on tape for 20–30 s with a TV camera and videotape recorder Later it was determined from the videotape whether the cell group was coordinated or not

B *Methods using photoamplifier and chart-recorder* In support of visual observation equipment was also used by which contractions of cell group could be registered A sensitive photoamplifier was connected to the examination chamber (0 15 ml made on a slide) The cell group in HM was placed on the bottom of the chamber between the tips of two glassfibres (Ø 0.05 mm) on a straight line cold light beam through one fibre passed the cleft with the cell group and was transmitted to a sensitive photoamplifier through another fibre Contractions in the cell group changed the intensity of the light The signal from the photoamplifier was displayed on a chart-recorder The cell group was placed between the fibre tips with a thin glass capillary either by hand or using a micromanipulator After 4–7 min caffeine was added with a micropipette (5 μl) The test temperature was 20°C (room temperature)

Contraction velocity

After incubating the cell groups in a test tube for 30 min at 15°C the test tube was warmed to 20°C and a sample of cell groups was placed in a depression slide as described above The microscope heating stage was adjusted to the testing temperature (20°C 30°C or 35°C). A coordinated cell group was searched for and its contractions were recorded on a videotape for about 1 min. The contraction velocity was measured on a TV screen from a typical contraction wave in each cell group with the aid of an ocular scale and a stop-watch The time intervals for measurement were about two seconds

Statistics

The difference in coordination vs uncoordination was tested with the 2×2 contingency table test and the coordination velocity difference was tested with the *t*-test The limit of significance was chosen as 0.05

RESULTS

Effects of time and temperature on the coordination

As stated previously (Pelkonen & Tirri 1972) single cells of a cell group usually contract in an uncoordinated way i e each muscle cell according to its own rhythm In some cases however the action of the cell group becomes coordinated so that one cell served as a pacemaker starting the waves of contractions We now observed that sometimes there may be two or more pacemaker cells in the group functioning alternatively We also found that these uncoordinated contractions become more regular with time when the cell groups are incubated in HM (Fig 1) The figure further shows that this spontaneous coordination of contractions is temperature-dependent Immediately after the preparation of the cell group in the ice-cold HM all the cell groups functioned in an uncoordinated way The number of the coordinated cell groups increased almost linearly with time of incubation so that after 90 min at 15°C all the cell groups studied were functioning in a coordinated way However only 75 % of the cell groups were coordinated when the

not observe any clear contracture in our experiments using azide concentrations up to 50 mM. The inhibition of spontaneous coordination by azide could be best explained by findings that azide inhibits Ca^{2+} release from the sarcoplasmic reticulum (Entman et al. 1973).

If we assume that the Ca^{2+}-induced Ca^{2+} release initiates the contraction, the question arises whether the contaminative Ca^{2+} in HM is sufficient to induce this. It is observed that only ~4 μM concentration of outside Ca^{2+} is needed for the half-maximal stimulation of Ca^{2+} release from microsomes (Kirchberger & Wong 1978). In our experiments Ca^{2+} concentration in the HM was not determined, but there are determinations from similar media indicating Ca^{2+} concentrations of about 8–9 μM (Bloom & Mulholland 1976, Bloom 1978). At a steady state of Ca^{2+} influx and efflux it was estimated to be about 1 mM (Bloom 1978), which is still far more than the level of 10^{-7} M that has been found to cause a cyclical Ca^{2+} release and uptake in skinned cardiac cells (Fabiato & Fabiato 1975 *a*).

A possible sequence of events may be posited concerning the rhythmic contractions which spontaneously become coordinated. At first the beating could be totally dependent on extracellular contaminative Ca^{2+}. This activates the myofilaments without participation of Ca^{2+} from the sarcoplasmic reticulum. The contractions are uncoordinated. A possible concomitant Ca^{2+} release from the sarcoplasmic reticulum would be masked by this extracellular Ca^{2+}. Later when Ca^{2+} uptake has proceeded, the role of the sarcoplasmic reticulum Ca^{2+} as a contraction inductor is increased. This Ca^{2+} is then able to diffuse to other cells initiating the coordinated type of contractions in a cell group. This would occur as a Ca^{2+}-induced Ca^{2+} release which is known to function cyclically (Fabiato & Fabiato 1975*a*). The presence of extracellular Ca^{2+} is, however, needed for the repeated activation of the Ca^{2+} release.

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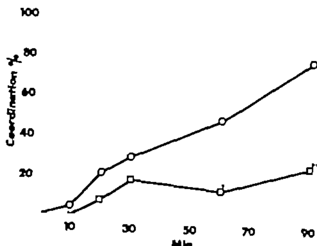


Fig 4 The effect of azide on the coordination of the cell groups at 10°C At every incubation time (abscissa) the percentage of coordinated cell groups (ordinate) is calculated In the azide experiments 1 –3 cell groups at every incubation time (□—□) and in the controls 3–35 cell groups at every incubation time (O–O) were used The azide concentration was 5×10 M The points marked with asterisks differ significantly from the controls ($P<0.01$)

Velocity of the contraction wave

The velocity of the contraction wave was measured at 3 temperatures at 20°C at 30°C and at 35°C It was surprising that the elevation of temperature had no effect at all on the velocities of the contraction waves The results obtained were 90±7 μm/s ($n=1?$) 100±6 μm/s ($n=11$) and 100±7 μm/s ($n=3$) respectively

DISCUSSION

The results show that the contractions in the myocardial cell group become coordinated in 1–? h after the disaggregation so that usually one cell serves as a pacemaker This coordination occurs spontaneously when incubated in HM and it is dependent on temperature

Previously Pelkonen & Tirri (1972) suggested that the coordination wave was transferred electrically from cell to cell According to current thinking this is implausible no electrical activity can be observed in this ionic medium since the cells are depolarized moreover they can not be paced by a field stimulation (Bloom et al 1974) According to these and our own results the most obvious mediator of the contraction wave seems to be Ca Its release from the sarcoplasmic reticulum and binding to the troponin C are the final steps in the excitation-contraction coupling. When HM resembling intracellular fluid was used the induction of contractions starts at a later stage, by-passing the sarcolemmal electrical stages This idea explains why broken cells e g a half of a cell contracted as well as those with an intact sarcolemma.

A very slow speed (90–100 μm/s) of the contraction wave also indicates a nonelectrical type of events It has been observed that in a myocardial cell group with a disrupted sarcolemma the contraction wave initiated by the calcium-induced calcium release propagated at 80–100 μm/s (Fabiato & Fabiato 1975 *b*) This similarity may confirm that Ca^{2+} plays a conclusive role in propagating the contraction wave in a cell but probably also between the cells in these circumstances In the latter case this assumes that Ca^{2+} can quite freely diffuse through cell membranes especially through the intercalar discs Actually in some cases it seemed that the contraction propagated transversely through the ordinary sarcolemma from cell to cell

The spontaneous coordinations of contractions are clearly temperature-dependent an elevation of the temperature hastening the coordination Bloom (1970) observed that elevation of the temperature in the same conditions as we used has a positive chronotropic action on myocardial cells He also found that Ca^{2+} uptake was dependent of ATP and the temperature (Bloom & Mulholland 1976). The enzymatic mechanisms connected to Ca^{2+} uptake and release influx and efflux may in fact be involved in the coordination of contractions. It is surprising that the temperature had almost no effect at all on the speed of the contraction wave thus indicating a non-enzymatical event e.g. diffusion of Ca^{2+} in the propagation of the wave

It has been observed that caffeine increases the calcium induced calcium release when the sarcoplasmic reticulum is loaded over a certain level of calcium (Endo 1975) This could explain a stimulating effect of caffeine on the coordination The steady contracture followed when Ca uptake was not able to compensate an enhanced release due to greater concentration of caffeine in HM Azide has a depressing effect on Ca uptake into the mitochondria (Fanburg – Gergely 1965) There are also observations that azide can cause contracture of the skinned heart cells (Fabiato & Fabiato 1975 *a*) and an increase of resting tension in heart fragments (Bloom et al 1974) These effects suggest a greater free Ca level inside the cells. We could

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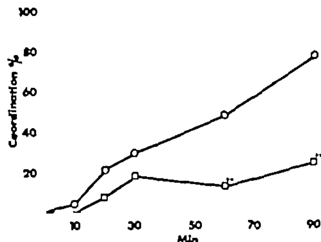


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Effect of intraluminal pH on the release of somatostatin and gastrin into antral bulbar and ileal pouches of conscious dogs

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UVNÄS-WALLENSTEN K, EFENDIC S, JOHANSSON C., SJÖDIN L. & CRANWELL, P Effect of intraluminal pH on the release of somatostatin and gastrin into antral, bulbar and ileal pouches of conscious dogs. Acta Physiol Scand 1980, 110 391–400 Received 11 March 1980 ISSN 0001-6772. Department of Pharmacology Karolinska Institute, Department of Endocrinology and Medicine Karolinska Hospital Stockholm, Sweden, Department of Drug Control, National Board of Health and Welfare Uppsala, Sweden and School of Agriculture La Trobe University Australia

Experiments were performed on conscious dogs with chronic pouches of the antrum, the duodenal bulb or the ileum, which were perfused with solutions of varying pH Gastrin and somatostatin levels were measured in the perfusates. When the pouches were perfused with 0 15 M NaCl only small amount of gastrin and somatostatin (1 pmol/min) were released into the lumen of the antrum and of the duodenal bulb By lowering pH of the perfusion fluid pH dependent release of somatostatin was induced into the lumen of the antrum and the duodenal bulb Perfusion with 0.1 M HCl caused large output of somatostatin (6–60 pmol/min) into the pouches. The upper pH limit for stimulation of the intraantral or intrabulbar somatostatin release appeared to be ~pH 3–4 Somatostatin was also released into ileal perfusates at intraluminal pH below 3–4 Lowering of pH in the antral pouches caused an increased intraluminal gastrin release, which was quantitatively less impressive than that of somatostatin. Occasionally also the gastrin release into the duodenal bulb increased during perfusion with 0.1 M HCl, whereas no such release was induced by acidification of the lumen of the ileum. It is suggested that the inhibition of gastrin release observed at low intraantral pH is mediated by a local effect of somatostatin, since this peptide is released in pH dependent manner in the antropyloric region It is also suggested that acidification of any region of the gastrointestinal tract will stimulate the release of peptides from all endocrine cells of the open type, probably by an unspecific effect on the membrane. Thus both gastrin and somatostatin are released by acidification of the antrum, but in the presence of high local levels of somatostatin, the release of gastrin is substantially inhibited

Key words Somatostatin gastrin, release, inhibition, antral pH intraluminal secretion

The antral mucosa is the main producer of the gastric acid stimulating hormone gastrin. Recently somatostatin, shown to inhibit gastric acid secretion, both by inhibiting gastrin release and by acting directly on the parietal cells (Bloom et al 1974 Le Roith et al 1975 Raptis et al 1975 Uvnäs-Wallensten, Efendic & Luft 1977) has been demonstrated in endocrine cells in the mucosa of the gastrointestinal tract. The antropyloric region is particularly rich in somatostatin containing D-cells some of which are located close to the gastrin containing G-cells (Rufener et al. 1975).

Acidification of the antrum and the proximal duodenum is known to inhibit gastric acid secretion. This effect is claimed to be in part mediated via a diminished output of gastrin (Andersson & Olbe 1963 Nilsson et al 1972 Csendes Walsh & Grossman 1972 Walsh, Richardsson & Fordtran 1975) and in part via release of inhibitory hormones, which block acid secretion at the

Table 1a and b pH in the pouch perfusates from the antrum ( ) and the duodenal bulb (b)

The pouches ere perfused ith 0 15 M NaCl during the first (0–120 min) and last (180–300 min) 2 h periods, i e. samples 1–4 and 7–10 During the midhours (in italics) the pouches were perfused ith 0 1 M HCl (pH ~1.2) or citrate phosphate buffer pH ,, 3, 4 or 5 Not the lag in changes of perfusate pH both hen pH is decreased and increased

Table 1

No of sample	Collection time (min)	0 1 M HCl pH 1	pH	pH 3	pH 4	pH 5
1	0	4 5	6.5	6 1	5.8	6.1
	30	6	6 7	6 0	6	5 9
3	60	6 3	6.7	6 0	4 8	5 6
4	90	6 3	6 7	5 9	5 7	5 6
5	120	4 3	*4.1*	*5.3*	*5.0*	*5 1*
		1	*2.5*	*3*	*4.0*	*5 1*
6	150	*1 4*	*2 4*	*3*	*4 0*	*5 1*
		1 3	*2.3*	*3*	*4.0*	*5 1*
7	180	1.5	4	3	4 1	5 1
		1.5				
8	210	8	4	3 1	4 1	5
		2 8				
9	240	4 4	4	3 6	4.3	5 3
		4 7				
10	270	4.7	5 4	4 5	5	5 7
		6 0				
	300					

Table 1b

No of sample	Collection time (min)	0 1 M HCl pH ~1	pH 2	pH 3	pH 3	pH 5
1	0	7 4	7 2	6.9	7.2	6.8
2	30	7	7 3	7.0	7.3	6 8
3	60	7 1	7 3	7.3	7 2	6 9
4	90	7 1	7 3	7 3	7.2	7 0
5	120	*2 6*	*4.3*	*3.8*	*7 1*	*6 8*
6	150	*1.6*	*2 6*	*3 1*	*5 1*	*5 8*
		1	*2 4*	*3 1*	*4 0*	*5 1*
7	180	*1.3*	*2 4*	*3 1*	*4 0*	*5 1*
		1 5	2 6	3.2	4.1	5 1
8	210	1 6				
9	240	3 8	3 4	3 5	4 6	6
10	270	5 6	4 9	4.9	5 7	6.2
	300	6 6	6 1	6.7	6 6	6 9

whereas pH of bulbar perfusates averaged ~7 The maximal decrease in pH was not obtained in antral or bulbar perfusates until ~30 min after starting the perfusion with the solutions of lower pH A similar delay until neutral pH was reached again, was observed during the reperfusion with 0 15 M NaCl. The lowest pH recorded during perfusion with 0 1 M HCl was ~pH 1.. in both antral and bulbar perfusates (Fig 1 and c)

The mean values ± S E. of the gastrin and somatostatin release per 30 min period at the dif ferent pH levels are depicted in Table a and b The values are based on the mean release obtained in each individual dog

Ant al perfu tes

Three dogs with chronic antral pouches were used in this series During the control period when the antrum was perfused with 0 15 M NaCl on the average 0 9 pmol/min of gastrin and 0 4 pmol/min of somatostatin were released into the lumen i e the ratio somatostatin/gastrin being 0.5/1. (These values are based on all control values n=.3..) (Table 2a)

After the control period either 0 1 M HCl (=13) citrate-phosphate-buffer pH .. (=18) pH 3 (=12) pH 4 (=4) or pH 5 (n=10) was introduced into the pouches. When perfusate pH was de creased an increase in somatostatin values was observed The lower the pH the more somatostatin appeared in the perfusates, during perfusion with 0 1 M HCl as much as 50 pmol/min was released. A small release of somatostatin was observed even at pH 4 (Table a Fig. 1).

A slight increase in gastrin output was also observed at the lowest pHs (F g. 1a Table a). How ever on a molar base the ratio somatostatin/gastrin was ~5 1 in these experiments (Fig. 1). When 0 15 M NaCl was reintroduced into the perfusion system somatostatin and gastrin values returned towards basal levels (Fig. 1a b and c)

Bulbar perf sat

Three dogs with bulbar pouches were used in this series The number of experiments performed at the different pHs were 0 1 M HCl (=8) pH 2.2 (=10) pH 3 (n=3) pH 4 (=) and pH 5 (n=2) Only very low amounts of somatostatin could be de tected in perfusates of the duodenal bulb during per fusion with 0 15 M NaCl (~0 1 pmol/min) (Tabl b) When the pH was lowered however a substantial increase of intraluminal release of somatostatin occurred. As in the antrum, perfusion with acid caused a large output of somatostatin (~20 pmol=min) The upper threshold for the initiation

parietal cell level (Thompson 1966 Wheeler 1974 Uvnäs 1971) Since somatostatin having both these properties is present in high amounts in the antropyloric mucosa it was tempting to investigate the possibility that the inhibitory effect on gastrin release and gastric acid secretion induced by low antral pH might be mediated through a release of endogenous somatostatin In support of this theory are the findings that D-cells degranulate during perfusion of the antropyloric lumen with acid (Fujita & Kobayashi 1971) and those demonstrating that somatostatin is released into the antral lumen of anesthetized cats in response to electrical vagal stimulation only during perfusion of the antrum with 0 1 M HCl whereas no such release occurs at neutral intraantral pH (Uvnäs Wallensten Efendic & Luft 1977) Furthermore portal vein somatostatin levels increase in anestethized dogs during instillation of acid into the stomach (Gustavsson & Lundqvist 1978 Shusdziarra et al 1978)

The aim of the present series of experiments was to investigate whether the inhibitory effect on gastrin release induced by acidification of the antrum is mediated by somatostatin Therefore we wanted to study the influence of pH in the antropyloric lumen on the release of gastrin and somatostatin For this purpose dogs were provided with chronic pouches of the antrum or of the duodenal bulb and these were perfused with solutions of varying pH

Somatostatin levels in plasma are difficult to measure and represent a mixture of somatostatin originating from several sources However gastrointestinal somatostatin is like gastrin released into the lumen of the antral and duodenal bulb (probably reflecting a bidirectional release of the peptide into the lumen and into the circulation) (Uvnäs-Wallensten & Rehfeld 1976 Uvnäs-Wallensten Efendic & Luft 1977 1978 Uvnäs-Wallensten 1977*a* 1978*a* Uvnäs-Wallensten & Efendic 1978) To circumvent these two problems we have chosen to study the release of gastrin and somatostatin by analyzing antral or bulbar perfusates for their content of these peptides

METHODS

Surgical procedures

Seven mongrel dogs weighing 16–21 kg were provided with various gastrointestinal pouches The dogs were allowed a period of 3 weeks for recovery after each operation

Series A In 3 dogs vagally innervated gastric fundic pouches (Pavlov type) were constructed by a slight modification of the method of Thomas (194_) At a second operation an antral pouch was isolated from the main stomach by the construction of a double mucosal wall after the antrum corpus border was visualized by the pH paper indicator technique (Andersson 1960, Olbe 1963). Gastrointestinal continuity was restored by gastrojejunostomy

Series B Four dogs were equipped with vagally innervated fundic pouches (Pavlov type). In a second operation a double mucosal wall was constructed in the pylorus in 3 of the dogs and gastrointestinal continuity was restored by gastrojejunostomy At a third operation the duodenum was transected just proximal to the entrance of the common bile duct The isolated bulb was anastomosed to a resected loop of the terminal ileum, which was brought out to the abdominal wall as a cutaneous fistula.

In the fourth dog, no pouch of the bulb was constructed A 10 cm long loop of the terminal ileum was isolated and one end of the loop was brought to the abdominal wall as a fistula.

Each experiment was performed after an 18 h fasting period Before the experiments a double lumen catheter was introduced into the pouch in order to allow perfusion Both antral bulbar and ileal pouches were perfused for 3 h with solutions at room temperature at a rate of 1–_ ml/min and at a very slight overpressure (10 cm H_2O). During the first and last h the pouches were perfused with 0.15 M NaCl During the mid third hour NaCl was exchanged for 0 1 M HCl or buffer solutions of pH 2 3 4 or 5 (McIlvain s standard buffer containing 0 1 M citric acid and 0 M NaH_2PO) –5 expts. were performed at each pH on each dog except as pH 3 4 and 5 in the dogs with bulbar pouches Due to the very small effects observed only a total of –3 expts. were performed at these pHs. On the dog with the ileal pouch only expts. at pH _2 3 and 4 respectively were performed

Treatment of sample

Samples of the pouch perfusate were collected in 15 or 30 min portions Immediately after collection, the pH of the perfusates was measured and adjusted to 7 by addition of 0 1 M NaOH using phenolphtalein as indicator The samples were heated to 100° for 1 min and were then kept frozen (–20°) until assayed for gastrin and somatostatin

Gastrin and somatostatin level were measured with radioimmunoassays as described by Nilsson (1975) and Efendic et al (1978) respectively

The release rate of the peptides (pmol/min) as calculated by multiplying the concentration of gastrin and somatostatin in the perfusates (pmol/ml) with the flow rate of the perfusate (ml/min)

RESULTS

pH of the pouch perfusates is depicted in Table 1*a* and *b* When the antral pouches were perfused with 0 15 M NaCl pH of the perfusates was ~6.

Table 2a and b *Influenc of pH on the release of gastrin (G) and somato tatin (S) (pmol/min ± S.E.) int pouch perfusates of the antrum (a) or the duodenal bulb (b)*

The pouches were perfused ith 0.15 NaCl during the first (0–120 min) and last (180–300 min) 2 h period, i.e. samples 1–4 and 7–10. During the midhour the pouches were perfused with 0.1 M HCl (pH ~1.2) or citrate phosphate buffer pH 3, 4 or 5 (in italics)

Table 2a

No. of sample	Collection time (min)		pH 1.2	pH 2.2	pH 3	pH 4	pH 5
1	0	G	893 237	649±85	817_51	827_194	406±115
		S	294 75	435 163	288 122	675_358	325±763
2	30	G	875_147	877_236	1 077_15	712±40	1 03_ _40
		S	26_ 79	451±110	348 193	799_102	996±268
3	60	G	1 229+ 46	1 029 4_	1 004 138	643 251	869±307
		S	180 47	498 221	470 701	307_197	794_223
4	90	G	940 772	946+106	917 797	541 81	683_13_
		S	205 92	359 171	649 310	155±75	176+99
5	120	G	*1 976_548*	*1 025±333*	*694±234*	*753_112*	*565±190*
		S	*5 490±3 043*	*1 399±518*	*849_507*	*615_455*	*215±141*
6	150	G	*7 810_3 618*	*1 1000±252*	*550±19*	*487±153*	*528+45*
		S	*36 49 18 787*	*5 654±5 574*	*2 783_1 715*	*1 008±868*	*2_1_76*
7	180	G	9 577±2 752	1 097±195	510±87	307±120	418±31
		S	52 984±23 890	6 702±3 673	354±1 477	677±457	177±72
8	210	G	4 375±484	845±93	374_82	379 140	371±81
		S	6 867±1 831	5 471±3 804	1 279±987	377±186	143±82
9	40	G	2 106±149	653±151	340±84	657±81	422±136
		S	2 452±645	1 367±571	1 073±932	282±164	147±100
10	270	G	2 253±234	989±90	367±188	424±152	384±163
		S	1 592±551	906 179	542±456	159_73	191±168
	300						

Table 2b

No. of sample	Collection time (min)		pH 1.2 8	pH 2.2 =10	pH 3 =8	pH 4 =	pH 5 =2
1	0	G	217±110	88 78	18±9.3	34	5
		S	103±7	65±25	112±59	60	52
2	30	G	323±235	56±49	26±13	0	7
		S	124±71	61±19	43±6	54	73
3	60	G	208±139	29±26	29±17	0	0
		S	55±14	58±3	31±12	19	88
4	90	G	365±80	11_10	29±17	0	11
		S	179±29	72±12	133±76	41	75
5	120	G	*543±262*	*50±21*	*15±8*	9	9
		S	*2 670±1 809*	*840±229*	*19 ±13*	*112*	*114*
6	150	G	*1 773±561*	*32±7*	*91±74*	*11*	*0*
		S	*21 165±5 648*	*1 282±598*	*472±76*	*180*	*112*
7	180	G	1 562±1 033	171±111	96±21	2	0
		S	17 568±3 128	1 795±1 233	421±143	230	86
8	210	G	993±705	132±72	12±9	20	0
		S	10 492±3 250	797±534	205±63	293	228
9	240	G	619±507	88±54	39±39	0	0
		S	1 842±465	122±39	112±13	181	130
10	270	G	557±390	33±12	33±19	7	0
	300	S	1 ±54	59±18	7±14	188	96

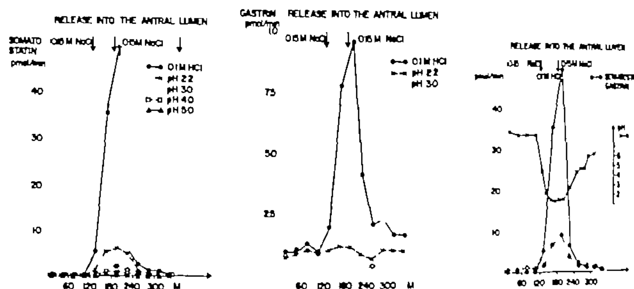


Fig. 1a. Influence of pH on the release of somatostatin (pmol/min) into the antral lumen of conscious antral pouch dogs. The pouches were perfused with 0.15 M NaCl for a h period at the beginning and end of each experiment. 1 h perfusion (between the arrows) with 0.1 M HCl or with buffer solutions of pH 2, 3, 4 and 5 was performed between the two control saline infusions.

Fig. 1b. Influence of pH on the release of gastrin (pmol/min) into the antral lumen of conscious antral pouch dogs. The experiments were performed as described in the text of Fig. 1a.

Fig. 1c. Influence of pH on the release of somatostatin and gastrin (pmol/min) during perfusion of the antral pouch with 0.1 M HCl. Note that the output of both gastrin and somatostatin peaks simultaneously, i.e. when the intra antral pH reaches its minimum level.

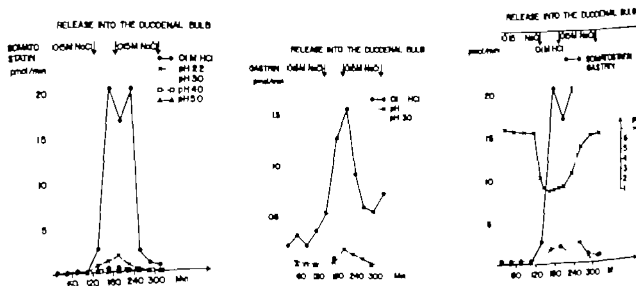


Fig. 2a. Influence of pH on the release of somatostatin (pmol/min) into the bulbar lumen of conscious duodenal bulb dogs. The experiments were performed as described in Fig. 1a.

Fig. 2b. Influence of pH on the release of gastrin (pmol/min) into the bulbar lumen of conscious duodenal bulb dogs. The experiments were performed as described in Fig. 1.

Fig. 2c. Influence of pH on the release of somatostatin and gastrin (pmol/min) on perfusion of the duodenal pouches with 0.1 M HCl. Note that the outputs of gastrin and somatostatin peak simultaneously, i.e. when the intrabulbar pH reaches its minimum level.

the immunoreactive material found in the antral and duodenal perfusates really corresponds to gastrin and somatostatin respectively.

As to the second question posed above, the following observations indicate that acidification of the antropyloric region in fact causes a secretion of somatostatin and not just a release due to the damage of mucosal cells. The increased appearance of somatostatin (and gastrin) observed during perfusion with 0.1 M HCl ceased when the pH of the perfusion medium was restored to neutrality again. This could not be the case if perfusion with HCl had induced an irreversible mucosal damage. Furthermore a significant output of somatostatin occurred into the antral and bulbar lumen also at pH 3–4, at which pH mucosal damage is not likely to occur. The reversed ratio between the somatostatin and gastrin amounts in neutral and acid perfusates is difficult to reconcile with the damage theory.

Also supporting the idea that low intraantral pH causes an active release of somatostatin and gastrin is the finding that peripheral venous somatostatin and gastrin levels increase in parallel with the intraluminal output of the peptides (Uvnäs-Wallensten et al., to be published). Thus low pH within the antrum or duodenal bulb causes the same bidirectional peptide release response just as do electrical vagal stimulations or injections of ACH, bombesin, apomorphine or sulphonamic drugs (Uvnäs-Wallensten 1977, Uvnäs-Wallensten, Efendic & Luft 1977, Uvnäs-Wallensten et al. 1978, Uvnäs-Wallensten, Lundberg & Efendic 1978, Uvnäs-Wallensten, Efendic & Johansson 1980).

What then is the physiological role of the pH dependent luminal release of somatostatin? In our view the hormones released into the gastrointestinal lumen may not necessarily exert any effects per se. The intraluminal secretion might be a spill over phenomenon reflecting high tissue levels of the peptides. The hormones might reach the lumen by intercellular diffusion, after having been released from the basal and lateral parts of the cells. For a more elaborate discussion on this matter see Uvnäs-Wallensten (1977, 1980), Uvnäs-Wallensten, Efendic & Johansson (1980).

Another interesting question is the possible physiological role of a pH controlled release of somatostatin within the antropyloric mucosa.

It is generally accepted that acidification of the antrum and proximal duodenum inhibits gastric acid secretion in two ways: by reducing the release of antral gastrin (for references see below) and by stimulating the release of inhibitory humoral agents such as antral chalones (Thompson 1966, Wheeler 1974) and bulbogastrone (Uvnäs 1971). The inhibitory effect of acid antral pH on stimulated gastrin release is well documented. Thus in dogs less gastrin is released in response to shamfeeding or insulin hypoglycemia when antral pouches are perfused with acid than when they are perfused with 0.15 M NaCl (Nilsson et al. 1972, Csendes, Walsh & Grossman 1972), but only few attempts have been made to quantitatively correlate the gastrin release in response to a certain stimulus to various intragastric pHs. However Walsh, Richardson & Fordtran (1975) found that in humans no gastrin release was obtained by amino acid stimulation when pH of the gastric content was maintained at pH≤2.5, whereas release responses of the same magnitude were obtained at pH 3–4 and 5.5. More detailed pH studies have been performed in earlier experiments on dogs in which gastrin release was measured indirectly as the rate of HCl secretion. Thus Andersson & Olbe (1963) showed that the shamfeeding induced acid secretion from a Pavlov Pouch was not reduced at intraantral pHs >2.5. A slight inhibition occurred at pH 2, whereas a substantial suppression of the HCl response was induced at pH 1–1.5.

Infusions of somatostatin mimick the effect of low antral pH on the release of gastrin. Thus infusion of somatostatin inhibits the release of gastrin induced by shamfeeding and insulin hypoglycemia in man (Konturek et al. 1976, Schrumpf et al. 1976, Raptis et al. 1975, Le Roith et al. 1975, Bloom et al. 1974), by feeding and by I.V. insulin and arginine in dogs (Barros, Bloom & Baron 1978, Ishida 1976) and by feeding and electrical vagal stimulation in cats (Albinus et al. 1975, and Uvnäs-Wallensten, Efendic & Luft 1977).

The results by Walsh, Richardsson & Fordtran, and by Andersson & Olbe are in good agreement with the hypothesis that the inhibition of antral gastrin release observed at low intraantral pH might be mediated by locally released somatostatin. Accordingly large amounts of somatostatin were released during perfusion of the antrum and the duodenal bulb with 0.1 M HCl and a profound inhibition of gastrin release and acid secretion was observed, whereas a still significant but tenfold lower somatostatin release was observed at pH

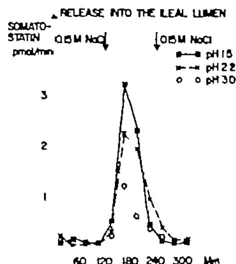


Fig 3 Influence of pH on the release of somatostatin (pmol/min) into an ileal pouch during perfusion with solutions of pH 1 5– and 3

of the somatostatin release was ~pH 3–4 (Fig 2*a* Table 2*b*)

A 2–3 fold increase of the intraduodenal gastrin release was also observed during perfusion with 0 1 M HCl (Fig 2*b*) the molar ratio somatostatin/gastrin being around 10 (Fig 2*c*)

Ileal perfusates

Two perfusions at pH 1 5 2 and 3 respectively were performed in a dog with an ileal pouch The perfusions were done exactly in the same way as in the dogs with antral and bulbar pouches We found that lowering the pH of the perfusion medium (to 1 5 2 2 and 3) caused a pH dependent increase of the intra ileal somatostatin release just as in the antral and in the bulbar pouches (Fig 3) No gastrin release was observed into the ileal lumen either at neutral or at lower pHs

DISCUSSION

In the present study we have shown that lowering of pH in antrum and duodenal bulb pouches causes a pH dependent output of somatostatin like immunoreactivity (SLI) into the lumen During the perfusion with 0 1 M HCl on the average 50 and 20 pmol/min were released into the antrum and the bulb respectively The upper pH threshold for activation of the somatostatin release appeared to be ~pH 3–4 A small but significant increase of the SLI also occurred into ileal perfusates during perfusion of the ileal pouch with solutions of pH 1.5–3

To our surprise the output of gastrin regular increased into antral and sometimes also into duodenal perfusates in response to intraluminal acidification However in comparison with the output of somatostatin the release of gastrin was small and only occurred on strong acidification i e during perfusion of the antrum with 0.1 M HCl

The occurrence of gastrin like immunoreactivity in antral perfusates of dogs is in agreement with studies performed by Andersson & Nilsson (1974) and by Rehfeld & Uvnäs Wallensten (1978). Furthermore both gastrin and somatostatin-like immunoreactivity has been shown to be released into the antral and duodenal lumen of cats in response to vagal stimulation and perfusion with alkaline solutions (Uvnäs Wallensten 1977 1978 1978*a* Uvnäs Wallensten & Efendic 1978 Uvnäs-Wallensten, Efendic & Luft 1977*a* 1978 Uvnäs Wallensten et al 1979) Human gastric instillates have also been shown to contain gastrin and somatostatin-like immunoreactivity (Knight et al 1978 Wisén et al in press)

Some questions may be raised however Do the immunoreactive materials observed in the *acid perfusates* really correspond to the two peptides in question and does acid pH increase the release of the peptides into the lumen by activating the inherent secretory mechanism? Or does acid pH just by damaging the mucosa lead to a high luminal content of gastrin and somatostatin deriving from exfoliated mucosal cells?

As to the first question The gastrin like immunoreactivity appearing in gastric juice and neutral antral perfusates of the cat has been identified as gastrin 17 by means of gelfiltration whereas antral perfusates of dogs have been shown to contain both gastrin 17 and 34 The corresponding types of gastrin are released into the circulation of cats and dogs respectively (Uvnäs Wallensten & Rehfeld 1976 Rehfeld & Uvnäs-Wallensten 1978) The somatostatin-like immunoreactivity has been characterized by Dr Pradayrol (Toulouse France) by combining radioimmunological technique and high pressure liquid chromatography He has been able to show that the SLI of antral and duodenal dog perfusates consists of the ordinary tetradecapeptide somatostatin and of a larger somatostatin molecule containing ~28 aminoacids in about equal proportions (to be published) The big type of somatostatin has been isolated from porcine intestinal mucosa (Pradayrol et al 1979) Thus it is highly likely that

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~2.2 at which pH level a much weaker inhibitory effect occurred (Andersson & Olbe 1963)

It cannot be established from the present data by which route the somatostatin reaches the G-cells If we assume that the intraluminal secretion reflects a high somatostatin concentration in the mucosa somatostatin might quite simply reach the G-cells by interepithelial diffusion the inhibition of gastrin release thus being an example of a paracrine mode of action However the axonlike protrusions of antral D-cells approaching the G-cells described by Larsson et al (1979) indicate that more specific anatomical relationships between somatostatin and gastrin cells might be involved in the inhibitory process

The mechanism by which HCl activates the release of somatostatin is not known Most endocrine cells of the antropyloric region are of the open type i e they have an ending provided with microvilli protruding into the lumen HCl possibly activates the release of somatostatin by acting directly on these microvilli of the D-cells Somatostatin was also released from cells in the ileum and the specificity of the HCl induced peptide release must therefore be questioned In fact several peptides are released in response to duodenal acidification Secretin and VIP are released into the bloodstream following instillation of acid into the duodenum (Schaffalitzky de Muckadell & Fahrenkrug 1978 Ebeid et al 1978) and small amounts of VIP and CCK are released by acid into the dog bulbar perfusates (Uvnäs-Wallensten & Fahrenkrug Uvnäs Wallensten & Rehfeld unpublished) It is possible that HCl activates the release of all peptides stored in endocrine cells of the open type (i e cells which are in direct contact with the lumen) The specificity of the pH controlled gastric acid inhibitory mechanism located to the antropyloric region might quite simply be determined by anatomical restrictions as to where a low pH may occur together with endocrine cells of the open type These criteria are only filled in the antrum and duodenal bulb

Such a general activation of the peptide release from endocrine cells induced by acid might explain our apparently paradoxical finding of a gastrin release in response to acidification of the antrum Maybe 0.1 M HCl activates gastrin release as potently as it activates somatostatin release but the smaller gastrin release observed in comparison to the output of somatostatin inspite of the larger population of G-cells than of D-cells might be du to the fact that the G-cells are largely inhibited b the high local levels of somatostatin

In this context it is of interest to mention, th the ratio between the amounts of somatostatin an gastrin found in human gastric instillates (slight alkaline) was 4-fold higher in patients with chron peptic ulcer disease than in controls (Wrén et al in press) Thus the rate of release of these peptide and maybe also the rate of peptide synthesis migh be affected by longstanding variations of the intr antral pH

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Pre- and postjunctional effects of prostaglandin E_2, prostaglandin synthetase inhibitors and atropine on cholinergic neurotransmission in guinea pig ileum and bovine iris

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GUSTAFSSON L, HEDQVIST P & LUNDGREN G. Pre- and postjunctional effects of prostaglandin E_2, prostaglandin synthetase inhibitors and atropine on cholinergic neurotransmission in guinea pig ileum and bovine iris. Acta Physiol Scand 1980 110: 401–411. Received 12 March 1980. ISSN 0001-6772. Department of Physiology and Department of Toxicology, Karolinska Institutet, Stockholm, Sweden.

The effects of prostaglandin E_2, arachidonic acid, prostaglandin synthetase inhibitors and atropine on cholinergic neuromuscular transmission were examined in isolated guinea pig ileum longitudinal muscle and bovine iris sphincter muscle. Prostaglandin E_2 and arachidonic acid markedly enhanced contraction responses induced by nerve stimulation. In addition, prostaglandin E_2 enhanced contraction responses to acetylcholine and direct muscle stimulation, approximately to the same extent as those to nerve stimulation. Prostaglandin synthetase inhibitors (indomethacin, meclofenamic acid and eicosatetraynoic acid) very effectively reduced contraction responses to nerve stimulation or acetylcholine and they annulled the stimulant effect of arachidonic acid. Basal and nerve-induced efflux of acetylcholine from the exercised tissues, as measured by mass fragmentography, was unaltered by prostaglandin E_2 but diminished slowly during indomethacin. Subsequent prostaglandin administration caused a slight increase of nerve-induced release of acetylcholine. Atropine markedly increased overflow of acetylcholine form stimulated preparations of both types. This indicates that muscarinic receptors, causing diminished acetylcholine release in exercised tissue, may be present also at postganglionic terminals. During atropine, an effect of prostaglandin E_2 on acetylcholine release could still not be seen. Thus, using physicochemical determination of acetylcholine we could not verify earlier reports, employing bioassay, claiming enhanced release of transmitter during prostaglandin E_2 treatment. However, it seems likely that prostaglandin E_2 takes part in the regulation of contractility and tone of the smooth muscle cells.

Key words: Acetylcholine, atropine, bovine iris, choline, gas chromatography–mass spectrometry, guinea pig ileum, neuromuscular transmission, prostaglandins, smooth muscle

Prostaglandins (PGs) of the E and F series contract and PG synthesis inhibitors relax smooth muscle in several cholinergically innervated tissues, such as the intestine and iris (Bergström et al. 1959, Bennett, Eley & Willis 1972, Ferreira, Herman & Vane 1972). Since PGs are released from these tissues, both basally and in response to a variety of stimuli (Posner 1973, Botting & Salzmann 1974), a possible role of locally formed PGs as modulators of smooth muscle tone has been envisaged (Bennett & Posner 1971, Ferreira, Herman & Vane 1976).

Apart from directly stimulating the smooth muscle cells, PGs may also influence the cholinergic neuroeffector transmission in guinea pig ileum (Harry 1968) and bovine iris (Gustafsson, Hedqvist & Lagercrantz 1975). Since PGs enhance contraction responses both to acetylcholine (ACh) and nerve stimulation (Harry 1968, Baum & Shropshire 1971, Hadházy, Illés & Knoll 1973, Illés, Vizi & Knoll 1974, Gustafsson et al. 1975) a postjunctional potentiating effect on cholinergic transmission may be present, although results denying this have been presented (Hall, O'Neill & Sheehan 1975).

The hazards in interpretation of differential quan-

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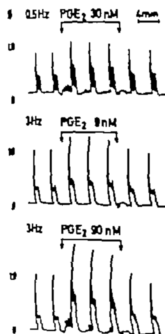


Fig. 1. Isolated longitudinal muscle of the guinea pig ileum. Effect of PGE_4 on phasic and tonic contraction responses to transmural stimulation (0.3 or 3 Hz, 1 ms) for 1 min at 4 min intervals. Wash after each application of the prostaglandin. Isometric recording.

effect was rapid in onset, vanished quickly when the preparation was washed, and occurred with PGE_4 concentrations (3–100 nM) and stimulation frequencies (0.5–3 Hz) used when release of acetylcholine (ACh) was determined. In both tissues PGE_4 potentiated contraction responses to ACh, as well as those induced by direct muscle stimulation after prior treatment with tetrodotoxin (0.1–0.3 μM) (Figs. 2, 3). These results strongly suggest that PGE_4 enhanced also neurogenic responses by a postjunctional action. In fact, PGE_4 (0.6 nM) increased the contraction response to direct muscle

Table 1. *Percent increase of contraction responses to transmural nerve stimulation (3 Hz, 1 ms, 15 pulses at 1 min intervals) 10 min after administration of arachidonic acid (AA) in bovine iris sphincter*

Mean values ± S.E. from 5 expts. at each dose level. All values significant at least at the 5% level.

AA μM	% increase
10	29 ± 9
30	46 ± 16
100	11 ± 33

stimulation in the bovine iris by 47 ± 15% ($P<0.05$, $n=6$) which does not differ from previously observed enhancement of neurogenic responses in this tissue (cf. Gustafsson et al. 1975).

The prostaglandin precursor arachidonic acid dose-dependently enhanced the contraction response to transmural nerve stimulation in guinea pig ileum and bovine iris sphincter (Table 1). Administration of either of two prostaglandin synthetase inhibitors meclofenamic acid and indomethacin (? 3 μM) annulled the effect of arachidonic acid without blocking the stimulant action of PGE_2 (Figs. 4, 5). This indicates that newly formed prostaglandin rather than arachidonic acid itself was responsible for the stimulant effect.

Indomethacin and meclofenamic acid (?–6 μM) as well as the substrate analogue eicosatetraynoic acid (ETA) (10–70 μM), depressed the contraction response to transmural nerve stimulation (Figs. 4, 6, 7). They also depressed the contraction response to exogenous ACh (Fig.). Inhibition of neurogenic responses developed rapidly in the guinea pig ileum being manifest within 30 s and reaching its maximum after 5–7 min, whereas the effect developed at a somewhat slower rate in the iris. In both tissues the end result was, however, the same.

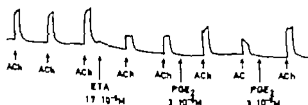


Fig. 2. Isolated bovine iris sphincter muscle. Reversal by PGE_4 of the inhibitory effect of eicosatetraynoic acid (ETA) on contraction responses to standard dose (3.3 μM) of acetylcholine (ACh). Wash at dots.

titative effects of PGs on the responses to exogenous agonist and to nerve stimulation respectively have been emphasized (Baum & Shropshire 1971). However inferring form smaller effect on ACh responses than on responses to transmural stimulation PGs have been claimed to enhance and PG synthesis inhibitors to decrease transmitter release from cholinergic nerves in guinea pig ileum (Ehrenpreis Greenberg & Belman 1973 Kadlec Masek & Seferna 1974 Bennett Eley & Stockley 1975)

Attempts to disclose a prejunctional effect of PGs on cholinergic neurotransmission by measurement of ACh release have led to conflicting results. Thus on the basis of bioassay of released ACh PGs have been reported to lack effect (Hadházy et al 1973 Illés et al 1974) or to enhance (Radmanovic 1975 Kadlec Masek & Seferna 1978) the secretion of transmitter in response to nerve stimulation. Likewise PG synthesis inhibitors have been reported either to depress (Kadlec et al 1974 Hall et al 1975) or to have no effect (Botting & Salzmann 1974 Hazra 1975) on induced secretion of ACh

In view of these conflicting results it was decided to re-investigate pre and postjunctional effects of PGs and PG synthesis inhibitors on cholinergic neurotransmission using gas chromatography–mass spectrometry (GcMs) for determination of secreted ACh and choline. Experiments were performed with both the guinea pig ileum and the bovine iris sphincter to differentiate between pre and postganglionic mechanisms. It was further of interest to make experiments taking in account a possible auto-inhibition of ACh release in eserinized tissue (cf Szerb 1979) and experiments were thus also performed in the presence of atropine. A preliminary account of part of the results has been presented elsewhere (Hedqvist et al 1980)

METHODS

General procedure

Male guinea pigs (250–500 g) were stunned and bled. Strips of plexus-containing longitudinal muscle from the distal part of ileum were isolated according to Paton & Vizi as described previously (Gustafsson et al 1978). Whole segments of ileum with tied ends were prepared as well and stimulated according to Finkleman (Gustafsson et al 1978) for activation of mesenteric adrenergic nerves. Also used was the isolated sphincter muscle from bovine irides (Gustafsson et al 1975). The smooth muscle preparations were mounted in 5 ml organ baths containing Tyrode s solution of the following composition (conc in mM) Na 149 2 K 2.7 Ca 1.8 Mg 0.5 Cl 144 2 H_2PO HCO$_3$ 11 9 and glucose 5.5. In some ileum experim the Tyrode s was replaced by a Krebs solution accor to Kadlec et al (1974) (conc in mM Na 135 4 K 5.9 2.5 Mg 1.2 Cl 133.3 HCO$_3$ 15 4 and glucose 11.5) bath fluid was continuously gassed with 5% CO_2 in O_2 was kept at 37°C. Biphasic single pulses or trains of pu (duration 1 msec supramaximal voltage specifically tivating the nerves) were delivered by means of a pa platinum wire electrodes along the walls of the bath, a Grass S88 stimulator. Muscle tone and contractions duced by drugs and transmural nerve stimulation studied at a load of 0.2–0.5 g and recorded ei isotonically (Harvard Apparatus smooth muscle tr ducer type 356) or isometrically (Grass FT 03C tr ducer) and displayed on an ordinate writer (Harvard E tronik 194) or a polygraph (Grass 5P). Figures s isotonic recordings unless otherwise stated

Release of ACh from the preparations was determ essentially as described by Gustafsson et al (1978). B ly the preparation was mounted in a 1 ml bath perifused with Tyrode s containing 3 μM eserine preparation was transmurally stimulated (0.5–3 H msec supramaximal voltage) for 5 min at 20 min inter Muscle tone and induced contractions were reco isometrically. The bath fluid was collected at 5 min i vals immediately chilled acidified and stored at until analyzed for ACh and choline by GcMs accord the method of Karlén et al (1974)

Drugs

Acetylcholine hydrochloride (Sigma Co St. L USA) arachidonic acid (Sigma) atropine sulfate (Sig eicosatetraynoic acid (F Hoffmann-La Roche & Basle Switzerland) eserine sulfate (Sigma), gua dine sulfate (Ciba-Geigy AG Basel Switzerland), 6- dopamine (Sigma), indomethacin hydrochloride (Me Sharp and Dohme Rahway New Jersey USA), pr glandin E free acid (a gift from Dr J Pike The Up Co Kalamazoo Michigan USA) sodium meclo mate (Parke Davis & Co Detroit Michigan, U tetrodotoxin (Sigma). Deuteriated (D_4) ACh and ch derivatives were synthetized at the Department of cology Karolinska Institutet. All chemicals used of reagent grade

Statistics

Experimental data were expressed as means ± S.E. St tical significance was tested according to Student's *t* for paired or unpaired variates

RESULTS

In harmony with previous results (Baum & Sh shire 1971 Gustafsson et al 1975) PGE$_2$ enhan the twitch response to transmural nerve stimula in guinea pig ileum and bovine iris sphincter. T contractions induced by prolonged stimula periods were also enhanced by PGE$_2$ (Fig 1)

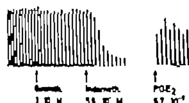


Fig 6 Isolated longitudinal muscle of the guinea pig ileum Contraction responses to transmural stimulation (5 Hz, 8 pulses, 1 ms) at 1 min intervals. Reversal by PGE2 of the inhibitory effect of indomethacin during adrenergic block with guanethidine

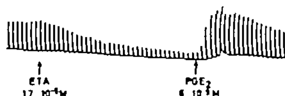


Fig 7 Isolated bovine iris sphincter muscle Contraction responses to transmural stimulation (3 Hz, 15 pulses, 1 ms) at 1 min intervals. Reversal by PGE2 of the inhibitory effect of indomethacin.

active as inhibitors of neurogenic responses in the presence of the adrenergic neurone blocker guanethidine (3 μM). This occurred independently of whether normal Tyrode's or the Kreb's solution of Kadlec et al was used (Fig. 6). In the above mentioned concentration guanethidine blocked completely the adrenergic transmission in the guinea pig ileum prepared according to Finkleman. Furthermore, in two experiments guinea pigs were chemically sympathectomized (Sachs & Jonsson 1975) with 6-hydroxy-dopamine (25 mg/kg intracardially on two consecutive days). This treatment reduced cardiac catecholamine levels by at least 90%. Yet, indomethacin inhibited the nerve induced contraction response in the ileum exactly as in preparations from untreated animals.

In the non-eserinized guinea pig ileum transmural nerve stimulation, 3–10 Hz for 5 min in the absence or presence of atropine (1 4 μM) evoked a minute outflow of ACh (less than 1 pmol/ml) which could not be accurately quantified by GcMs. Basal outflow of ACh was always below the level of detection

In the eserinized tissue (3 μM) transmural nerve stimulation evoked a reproducible and frequency dependent outflow of ACh well above the resting level (Fig 9 Table) Choline also accumulated in the bath medium but nerve stimulation did not change the rate of accumulation (Table 2) Notably virtually the same amounts of choline were found in the medium surrounding the non-eserinized ileum and again there was no difference between resting and stimulation periods.

Addition to the bath of atropine in concentrations ranging between 14 nM and 1 4 μM consistently and dose-dependently increased the stimulation-evoked release of ACh A further increase in atropine concentration to 14 μM did not cause any additional

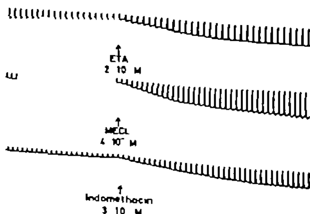


Fig 8 Isolated bovine iris sphincter muscles. Contraction responses to transmural stimulation (3 Hz, 15 pulses, 1 ms) at 1 min intervals After more than one hour at 37°C tone had developed spontaneously and was effectively reversed by eicosatetraynoic acid (ETA), meclofenamic acid (MECL) or indomethacin.

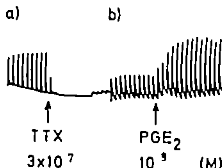


Fig 3 Isolated bovine iris sphincter Potentiation by PGE_2 of contraction responses to transmural stimulation during tetrodotoxin (TTX) treatment (*a*) 3 Hz, 1 ms 15 pulses at 1 min intervals (*b*) 40 ms pulse duration contractions amplified ×4

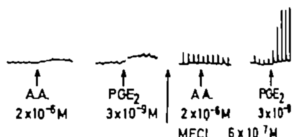


Fig 4 Isolated longitudinal muscle of the guinea p ileum Contraction responses to transmural stimulati (1 Hz 2 pulses, 1 ms) at 1 min intervals. Effect arachidonic acid (A A) or PGE_2 before and during a ministration of meclofenamic acid (MECL). Wash b tween registrations

i e a very marked or virtually complete inhibition of the contraction response which was highly resistant to washing of the preparation Subsequent administration of PGE_2 in very low concentrations (0 6–6 nM) promptly increased the contraction response to transmural nerve stimulation (Figs 4 6 7) Using slightly higher concentrations of PGE_2 (10–60 nM) normal or supranormal responses were consistently obtained

A conspicuous feature of the bovine iris sphincter is a progressive rise in intrinsic tone occurring after approximately 1 h of incubation in Tyrode s solution (Gustafsson et al 1975) The increase in tone seems to be parallelled by prostaglandin accumulation in the bath medium (Posner 1973) In the present study anyone of three prostaglandin synthetase inhibitors indomethacin meclofenamic acid and eicosatetraynoic acid annulled the expected rise in tone They also brought an alread developed tone back to its basal level (Fig 8), sub sequently followed by inhibition of the nerve induced contraction responses These observation suggest that endogenous PGE_2 may modulate bot intrinsic tone and the neuroeffector transmission and that the latter action requires lower concen trations of the compound This view is supporte by the effects of exogenous PGE_2 low doses en hancing contraction responses and higher doses in creasing the intrinsic tone

It has been suggested that indomethacin inhibit contraction responses in the guinea pig ileum b causing increased release of noradrenaline in tur inhibiting the ACh release and leading to reduce effector responses (Kadlec et al 1974) We hav consistently failed to confirm this view Thus in domethacine and meclofenamic acid remained full

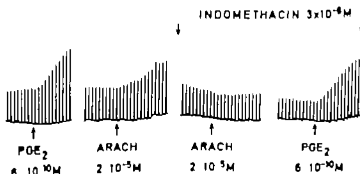


Fig 5 Isolated bovine iris sphincter muscle Contraction responses to transmural stimulation (3 Hz 15 pulses 1 ms) at 1 min intervals Effect of PGE_2 or arachidonic acid (ARACH) before and during administration of indomethacin Wash at dots.

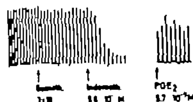


Fig. 6 Isolated longitudinal muscle of the guinea pig ileum. Contraction responses to transmural stimulation (5 Hz, 10 pulses, 1 ms) at 1 min intervals. Reversal by PGE₂ of the inhibitory effect of indomethacin during adrenergic block with guanethidine.

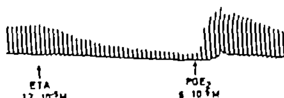


Fig. 7 Isolated bovine iris sphincter muscle. Contraction responses to transmural stimulation (3 Hz, 15 pulses, 1 ms) at 1 min interval. Reversal by PGE₂ of the inhibitory effect of indomethacin.

active as inhibitors of neurogenic responses in the presence of the adrenergic neurone blocker guanethidine (3 μM). This occurred independently of whether normal Tyrode's or the Kreb's solution of Kadlec et al. was used (Fig. 6). In the above-mentioned concentration guanethidine blocked completely the adrenergic transmission in the guinea pig ileum prepared according to Finkleman. Furthermore in two experiments guinea pigs were chemically sympathectomized (Sachs & Jonsson 1975) with 6-hydroxy-dopamine (25 mg/kg intracardially on two consecutive days). This treatment reduced cardiac catecholamine levels by at least 90%. Yet, indomethacin inhibited the nerve induced contraction response in the ileum exactly as in preparations from untreated animals.

In the non-eserinized guinea pig ileum transmural nerve stimulation, 3–10 Hz for 5 min in the absence or presence of atropine (1.4 μM) evoked a minute outflow of ACh (less than 1 pmol/ml) which could not be accurately quantified by GcMs. Basal outflow of ACh was always below the level of detection.

In the eserinized tissue (3 μM) transmural nerve stimulation evoked a reproducible and frequency dependent outflow of ACh well above the resting level (Fig. 9, Table 1). Choline also accumulated in the bath medium but nerve stimulation did not change the rate of accumulation (Table 2). Notably virtually the same amounts of choline were found in the medium surrounding the non-eserinized ileum, and again there was no difference between resting and stimulation periods.

Addition to the bath of atropine in concentrations ranging between 14 nM and 1.4 μM consistently and dose-dependently increased the stimulation-evoked release of ACh. A further increase in atropine concentration to 14 μM did not cause any additional

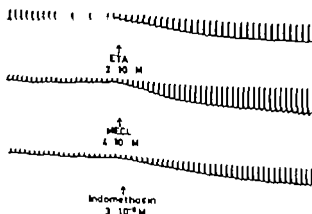


Fig. 8 Isolated bovine iris sphincter muscles. Contraction responses to transmural stimulation (3 Hz, 15 pulses, 1 ms) at 1 min intervals. After more than one hour at 37°C tone had developed spontaneously and was effectively reversed by eicosatetraynoic acid (ETA), meclofenamic acid (MECL) or indomethacin.

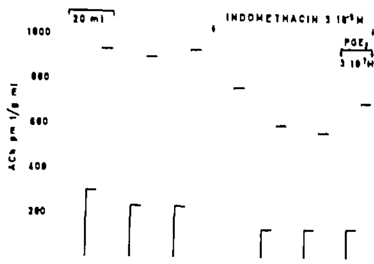


Fig 9 Effect of indomethacin and PGE₁ on acetylcholine (ACh) release as measured by GcMS from isolated longitudinal muscle of the guinea pig ileum Transmural stimulation (3 Hz 1 ms 840 pulses) during 5 min periods at 20 min intervals. Bath fluid changed every 5 min Short columns pre stimulation periods tall columns stimulation periods

increase in ACh release (Fig 10) Atropine 1 4 μM substantially increased the release of ACh at both 0 5 and 3 Hz stimulation whilst having no effect on basal release of the transmitter (Table 2) The nominal increase in ACh release was more marked at 3 Hz stimulation but on a percentage basis the enhancement was virtually the same being 210 and 184 % at 0 5 and 3 Hz respectively Atropine did not significantly affect choline release from the resting or stimulated guinea pig ileum (Table 2)

Closely similar results were obtained in 3 expts with the bovine iris sphincter Transmural nerve stimulation at 3 Hz released 97±42 pmol ACh × g⁻¹× min⁻¹ Subsequent administration of atropine 1 4 μM increased the release of ACh to 285±91 pmol × g⁻¹× min⁻¹ during the following stimulation period

Administration of PGE₁ 10 min before and during a subsequent 5 min stimulation at 0 5 or 3 Hz was found to have no effect on basal and stimulation evoked release of ACh in the guinea pig ileum This lack of effect was seen both with a PGE₁ concentration 3 nM which normally gives rise to marked enhancement of stimulation-evoked contraction responses (cf Fig 1) and a 100 times higher concentration (Table 3 Fig 11) Choline release was equally unaffected by PGE₁ in these concentrations In the bovine iris sphincter (2 expts) PGE₁ 0 3 μM had no discernible effect on the release of ACh induced by 3 Hz stimulation irrespectively of being tested before or after atropine treatment 1 4 μM

In view of indomethacin effectively inhibiting the contraction response to nerve stimulation its effect

Table 2 *Isolated longitudinal muscle of the guinea pig ileum*

Effect of 1 4 μM atropine on acetylcholine (ACh) and choline (Ch) release immediately before (basal) and during (stim) transmural nerve stimulation (0 5 or 3 Hz 1 ms, 140 or 840 pulses respectively) Bath fluid collected at 5 min intervals ACh and Ch determined by GcMS and expressed in pmol/g × min (tissue wet weight) (Mean values ± S E * $P<0.05$ ** $P<0.001$ *n* = number of expts)

	Control		Atropine 1 4 μM		
	ACh	Ch	ACh	Ch	*n*
Basal	166± 11	1 017±168	148± 51	903± 5	4
0 5 Hz stim	258± 33	1 139±–4	536± 85	935±200	4
Basal	228± 12	77 ± 95	13± 34	544±1–5	7
3 Hz stim	1 43±144	857± 71	2 285± 35	855±101	8

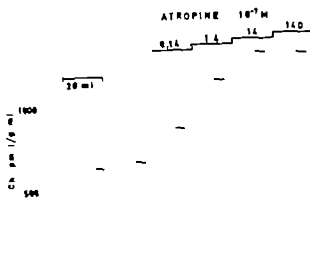


Fig. 10. Effect of atropine in increasing concentrations on stimulation-induced acetylcholine (ACh) release as measured by GcM from isolated longitudinal muscle of the guinea pig ileum. Transmural stimulation (3 Hz, 1 ms, 840 pulses) during 5 min periods at 20 min intervals. Bath fluid changed every 5 min.

on ACh release was also tested in the guinea pig ileum. Indomethacin, 30 μM reduced basal outflow of ACh as well as release of the transmitter induced by nerve stimulation at 0.5 and 3 Hz (Table 3 Fig. 9). The inhibition developed remarkably slowly reaching its maximum after 40 to 60 min, and it did not exceed 40%. In 4 out of 5 expts subsequent administration of PGE_2 0.3 μM caused a minor increase of ACh release during nerve stimulation.

Table 3. *Effect of PGE_2, atropine and indomethacin on GcMs-determined efflux of ACh induced by transmural nerve stimulation (0.5–3 Hz, 1 ms) for 5 min at 20 min intervals in guinea pig ileum longitudinal muscle*

Values are given as means ± S.E. in percent of control stimulation preceding drug administration. n = number of expts. Statistical evaluation according to Student's t-test for paired variates, in the case of PGE_2 effect in presence of atropine or indomethacin by setting atropine and indomethacin data to 100%. ATR = atropine, IND = indomethacin. n.s. = not significant.

Drug	0.5 Hz	3 Hz	0.5+3 Hz		
Nil	106±6 n=12	106±3 n=11	106±3	n.s.	
PGE_2 3 nM		111±12 n=3			
PGE_2 300 nM	105±9 n=5	107±6 n=3	106±6	n.s.	
ATR, 1.4 μM	210±28 n=4	189±14 n=8	196±13	$P<0.001$	n.s.
ATR+PGE_2, 1.4 μM+300 nM	200±45 n=4	193±11 n=3	197±24	$P<0.01$	
IND 30 μM	68±6 n=3	6.±6 n=3	65±4	$P<0.001$	$P<0.05$
IND+PGE_2, 30 μM, 300 nM	81±11 n=3	80 n=2	81±6	$P<0.05$	

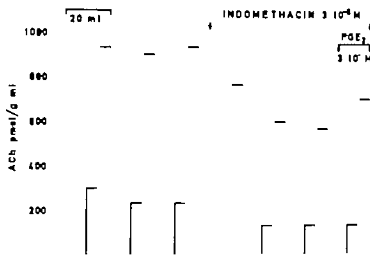


Fig 9 Effect of indomethacin and PGE_2 on acetylcholine (ACh) release as measured by GcMs from isolated longitudinal muscle of the guinea pig ileum. Transmural stimulation (3 Hz 1 ms 840 pulses) during 5 min periods at 20 min intervals. Bath fluid changed every 5 min Short columns pre-stimulation periods tall columns stimulation periods

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In view of indomethacin effectively inhibiting the contraction response to nerve stimulation its effect

Table ? *Isolated longitudinal muscle of the guinea pig ileum*

Effect of 1 4 μM atropine on acetylcholine (ACh) and choline (Ch) release immediately before (basal) and during (stim) transmural nerve stimulation (0.5 or 3 Hz, 1 ms 140 or 840 pulses, respectively) Bath fluid collected at 5 min intervals ACh and Ch determined by GcMs and expressed in pmol/g × min (tissue wet weight) (Mean values ± S E. $=P<0.05$ $=P<0.001$ $n=$ number of expts)

	Control		Atropine 1 4 μM		
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Basal	166± 11	1 017±168	148± 51	903±25	4
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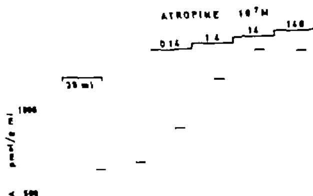


Fig 10 Effect of atropine at increasing concentrations on stimulation-induced acetyl choline (ACh) release as measured by GcMs from isolated longitudinal muscle of the guinea pig ileum. Transmural stimulation (3 Hz, 1 ms, 840 pulses) during 5 ml periods at 20 min intervals. Bath fluid changed every 5 min

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Values are given as means ± S.E. in percent of control stimulation preceding drug administration. n = number of expts. Statistical evaluation according to Student's t-test for paired variates in the case of PGE_2 effect in presence of atropine or indomethacin by setting atropine and indomethacin data to 100%. ATR = atropine, IND = indomethacin, n.s. = not significant

Drug	0.5 Hz	3 Hz	0.5+3 Hz		
NI	106±6 n=12	106±3 n=12	106±3	n.s.	
PGE_2, 3 nM		111±12 n=3			
PGE_2, 300 nM	105±9 n=5	107±6 n=3	106±6	n.s.	
ATR, 1.4 μM	210±28 n=4	189±14 n=8	196±13	P<0.001	n.s.
ATR+PGE_2, 1.4 μM, 300 nM	200±45 n=4	193±11 n=5	197±24	P<0.01	
IND 30 μM	68±6 n=3	62±6 n=3	65±4	P<0.001	P<0.05
IND+PGE_2, 30 μM, 300 nM	81±11 n=3	80 n=2	81±6	P<0.05	



Fig. 11. Effect of indomethacin and PGE_2 on acetylcholine (ACh) release as measured by GcMs from isolated longitudinal muscle of the guinea pig ileum. Transmural stimulation (3 Hz, 1 ms, 840 pulses) during 5 min periods at 20 min intervals. Bath fluid changed every 5 min. Short columns pre-stimulation periods, tall columns stimulation periods.

DISCUSSION

It has been suggested (Gustafsson et al. 1975) that prostaglandins might operate to modulate smooth muscle responsiveness to cholinergic nerve activity in the iris, the experimental basis for this assumption being that prostaglandins are formed in the tissue (Posner 1973), and that E and F prostaglandins cause a dose-dependent enhancement of contraction responses to cholinergic nerve stimulation (Gustafsson et al. 1975). The present results lend further support to this view. Thus, three prostaglandin synthetase inhibitors, indomethacin, meclofenamic acid and eicosatetraynoic acid, although chemically very dissimilar (cf. Flower 1974), inhibited the contraction responses to cholinergic nerve stimulation in the bovine iris sphincter muscle. Moreover, subsequent administration of PGE_2 restored normal contraction responses. In addition it was found that the prostaglandin precursor, arachidonic acid, enhanced nerve induced contraction responses, and that this effect was markedly counteracted, or even annulled, by prior application of indomethacin or meclofenamic acid.

In harmony with several other reports (Kadlec et al. 1974, Bennett et al. 1975, Ehrenpreis, Greenberg & Comaty 1976) PGE_2 enhanced and prostaglandin synthetase inhibitors reduced the contraction responses to cholinergic nerve stimulation in guinea pig ileum. Furthermore, the stimulant effect of PGE_2 was mimicked by arachidonic acid. Seemingly, PGE_2 could act on ganglionic and/or neuroeffector transmission in the ileum. However, the strikingly similar effects obtained in the iris sphincter, which is a ganglion-free preparation (cf. Beattie & Stilwell 1961), would seem to indicate that in both tissues the target was the neuroeffector transmission.

It has been proposed (Kadlec et al. 1974) that PGs enhance cholinergic responses in the guinea pig ileum by interfering with a suggested adrenergic control of ACh release (Benni, Bianchi & Crema 1969, Paton & Vizi 1969). The present results do not support such a mechanism, since PGE_2 and indomethacin remained fully active on cholinergic responses after adrenergic neuronal blockade (guanethidine) or chemical sympathectomy (6-OH-dopamine).

An important issue is whether the potentiating effect of PGs on the neuromuscular transmission is pre- or postjunctionally located. In both ileum and iris, PGE, enhanced and PG synthetase inhibition reduced contraction responses to ACh to approximately the same extent as they affected responses to nerve stimulation. In addition, PGE_2 potentiated responses to direct electrical muscle stimulation in the two preparations. Therefore, a substantial part of the PG effect must be due to a postjunctional action.

As pointed out in the introduction several reports have delt with a prejunctional action of PGs and PG synthetase inhibitors on ACh release in the guinea

pig ileum, and evidence has been presented indicating either that PGs enhance and PG synthetase inhibitors reduce ACh release or that in neither respect there is any effect on the release of ACh (Yadkiry et al 1973 Botting & Salzmann 1974 Illes et al 1974 Kadlec et al. 1974 1978 Hall et al 1975 Hama 1975 Ehrenpreis et al 1976) In all these studies ACh was measured biologically using the guinea pig ileum as assay tissue It is obvious that PGs and PG synthetase inhibitors have profound effects on the guinea pig ileum, and it is conceivable therefore that interference with the assay tissue could explain the divergent results in the above-mentioned studies In order to circumvent this possibly erroneous factor GeMs was used in the present study for identification and quantification of ACh released from the guinea pig ileum and the bovine sphincter

PGE$_2$ as tested over a wide range of concentrations and two stimulation frequencies were used (0.5 and 3 Hz) at which PGE_2 is known to have marked effects on induced contraction responses. Although this favourable conditions would seem to be present, we could not detect any effect of PGE_2 on ACh release from the guinea pig ileum or the bovine iris, irrespectively of whether the preparation is stimulated or not.

It is conceivable that eserine treatment, necessary for preservation of released ACh may mask an effect of exogenous PGE_2 on transmitter secretion. Thus eserine markedly raises muscular tone in guinea pig ileum and bovine iris in the former tissue followed by an increased PG synthesis (Botting & Salzmann 1974) Presently it is not known whether the same holds true for the iris but it is well established that an increase in tone is associated with an increased PG formation also in this tissue (Posner 1973) In both tissues the eserine-induced rise in muscular tone is effectively antagonized by atropine presumably leading t a decrease in PG formation, as has been demonstrated e.g. in bronchial tissue (Orehek, Douglas & Bouhuys 1975) It was decided therefore to test the *effect of PGE*$_2$ on ACh release in the presence of atropine or indomethacin, which also reduces muscular tone and which potently inhibits PG synthesis (Botting & Salzmann 1974)

After treatment of the guinea pig ileum and bovine iris sphincter with atropine in a concentration sufficient t annul the muscular effects of eserine PGE_2 was still ineffective in altering the nerve-induced efflux of ACh Notably atropine per se enhanced nerve induced release of ACh in guinea pig ileum and bovine iris indicating that transmitter secretion was not saturated in the presence of eserine. Previously it was shown that atropine increased the release of ACh induced by nerve stimulation or high potassium in the guinea pig ileum but not in the guinea pig heart and bladder or chicken heart (Kilbinger 1977 Sawynok & Jhamandas 1977 Kilbinger & Krieg 1978 Kilbinger & Wagner 1979). These observations were interpreted in terms of some tissues lacking prejunctional muscarinic receptors or a stimulant effect, when seen being due to blockade of inhibitory ganglionic muscarinic receptors (Kilbinger & Krieg 1978 Kilbinger & Wagner 1979) In the present study atropine increased ACh release equally effectively in guinea pig ileum and the ganglion-free bovine iris sphincter It may be concluded therefore that postganglionic cholinergic nerve terminals seem to possess muscarinic receptors possibly operated to feed-back control transmitter secretion

Indomethacin inhibited nerve-induced release of ACh in the guinea pig ileum as observed by others (Kadlec et al 1974 Hall et al 1975) In the presence of indomethacin PGE_2 tended to increase ACh release Seemingly these observations indicate that PGE_2 may have some st mulant effect on the release process at least when transmitter secretion is depressed by othe gents than ACh itself Even so the significance of PGE_2-mediated enhancement of ACh release seems rather unclear when considering the different effect of indomethacin or PGE_2 on ACh release in eserinized and on contraction responses in non-eserinized ileum Such a comparison is certainly warranted from the point of view that eserinization did not alter the efflux of PGE_2 during nerve stimulation of this tissue (Botting & Salzmann 1974) Thus the inhibitory effect of indomethacin on ACh release developed ery slowly and did not exceed 40% as compared to the rapid and almost complete inhibition of the contraction response Likewise the enhancement of ACh release by ubsequently administered PGE_2 was but small and developed slowly as compared to the rapid and usually complete recovery of contraction responses. It may be concluded therefore that PGE_2 mediated enhancement of ACh release if at all present, is of considerably less importance than its postjunctional effects.

The present results lend further support to the

concept of an at least in vitro endogenous PG mechanism modulating smooth muscle tone and contraction responses to cholinergic nerve stimuli at the postganglionic postjunctional level Evidence was not found for PGE_2 being a significant modulator of ACh release It was also found that atropine increased nerve induced release of ACh in guinea pig ileum and bovine iris in all probability by blocking postganglionic prejunctional muscarinic receptors

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Distensibility of left atrium in normotensive and spontaneously hypertensive rats

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RICKSTEN S E., YAO T LJUNG B. & THORÉN P Distensibility of left atrium in normotensive and spontaneously hypertensive rats. Acta Physiol Scand 1980 110 413–418. Received 13 March 1980 ISSN 0001-6772 Department of Physiology University of Göteborg, Sweden.

In spontaneously hypertensive rats (SHR) left atrial mechanoreceptors are reset. Thus, left atrial pressure must be almost twice as high in SHR as in normotensive rats to produce comparable degrees of receptor activation and reflex sympathetic inhibition. The present study was performed to investigate whether this resetting is due to decreased atrial distensibility in SHR. Static load-length relationships were therefore investigated on isolated left atrial strips from 11 pairs of male SHR and Wistar Kyoto rats (WKR). After each experiment the strips were fixed at passive tension of 4 mN and the average wall thickness was determined histologically Furthermore pressure–volume relationships were studied on non-beating, isolated left atria from SHR and WKR. Distensibility was here defined as % volume increase when LAP was increased from 2.5 to 12.5 mmHg either rapidly (0.5–1 s, dynamic distension) or slowly (3 min, static distension). Atrial wall thickness did not differ significantly in SHR and WKR, but the passive force (mN) per crossectional area exerted during elongation above 80% was greater ($P<0.05$) in SHR. Also the "dynamic" but not the "static" volume distensibility was significantly lower in SHR ($P<0.01$). The decreased dynamic distensibility of SHR left atrial walls can at least partly explain the resetting of the atrial receptors activated during the rapid filling phase

Key words. Left atrial mechanoreceptors resetting, atrial distensibility SHR.

Cardio-pulmonary receptors are spread throughout the heart, in the atria as well as in the ventricles, and also in the pulmonary veins (cf. Thorén 1979) The majority of these volume receptors fire in unmyelinated vagal afferents and activation of receptors induces reflex bradycardia and dilation of resistance and capacitance vessels, e.g. when blood volume is increased. Thorén et al (1979) found that the cardio-pulmonary receptors were reset in the spontaneously hypertensive rat (SHR) i e a greater atrial pressure was needed to activate these receptors in SHR (10 mmHg) compared to normotensive controls (~5 mmHg). Ricksten et al. (1979) showed that higher distending pressures in the left heart were needed in SHR to inhibit renal sympathetic nerve activity to the same extent as in normotensive controls. The mechanism behind this resetting of the cardiopulmonary stretch receptors in the hypertensive animal is not known. Receptor resetting can be due to a change in the receptor properties per se or/and to a structural change of the left atrial wall Aars (1969) showed that the resetting of the aortic baroreceptors in renal hypertensive rabbits was caused by a decreased distensibility of the aortic wall and not by a decreased sensitivity of the stretch receptor itself Other studies however indicate that alternations of the receptor properties can contribute to the resetting of the arterial baroreceptors in SHR at least in early stages in hypertension (Andresen et al 1978) The aim of the present study was to examine the distensibility of the left atrial wall where most of the left cardiac stretch receptors seem to be located in rats (Thorén et al 1979) further to explore whether decreased wall distensibility could explain the resetting of the left atrial receptors in SHR when compared to normotensive Wistar Kyoto rats (WKR)

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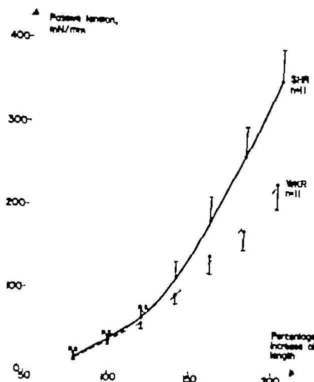


Fig 1 Static load–length relationship of isolated left atrial strips from SHR and WKR. Only at a *relative length* increase of more than 80% SHR show a greater passive tension per unit tissue area ($P<0.05$ $P<0.01$)

METHODS

Elastic properties of isolated left atrial wall For this purpose eleven pairs of male SHR weighing 302±3 g and having a mean arterial blood pressure (MAP) of 163±3 mmHg and Wistar Kyoto rats (WKR) with an MAP of 111±3 mmHg and a body weight of 308±3 g, were used MAP was measured some days before the experimental procedures via a tail artery catheter after recovery from brief ether anesthesia.

Under ether anesthesia the heart and lungs were excised from the rat and placed in ice-chilled Krebs solution After removal of the left auricle a standardized strip was excised from the lateral aspect of the left atrial wall with a width of 0.5–1 mm and a length of 3–4 mm The strip was tied at both ends with fine silk and mounted vertically between force transducer and a tissue support in an organ bath containing oxygenated Krebs solution at a temperature of 38°C Throughout the experiment the tissue was activated by electrical field stimulation at 3 Hz with pulses of 4 ms duration at supramaximal intensity During an initial accomodation period of 60 min the passive force was repeatedly adjusted to 4 mN At the end of this equilibration period the length of the tissue was determined by means of a Leitz dissection microscope with a micrometer eye piece and the value was defined as the reference length (100%) The strip was then stretched in steps of 0 2 mm at the early flat part of the length-tension curve and 0 1 mm at the steep part of this length-tension curve

At the end of the experiment the tissue was fixed at passive tension of 4 mN in Bouin's solution overnight After fixation the preparation was dehydrated in alcohol cleared in xylene embedded and sectioned perpendicularly to the longitudinal axis of the strip. Sections corresponding to the midpoint along the length of the atrial strip were selected and stained with Weigert's hematoxylin and van Gieson's method The sections were photographed in a Zeiss photomicroscope at 400× magnification. In all sections the myocardial fibres were cut transversely i e the fibers were orientated in the longitudinal direction of the tissue The total cross-sectional area of the magnified image of the strip was measured by means of a planimetric method The width of the tissue was determined at the well defined endocardial aspect of the cross-section. The average thickness of the strip was calculated by dividing the area value with the width of the left atrial strip The shape of the cross-sections was approximately rectangular

In each experiment the relationship between the percentage length increase of the strip beyond the reference length (strain) and the passive force per cross-sectional area (stress) was plotted Due to stress relaxation there was a decay in passive force at each increase in strain and the steady state value was used in the calculations Reduction of cross-sectional area of the strip during the elongation was not corrected for The difference between SHR and WKR was compared using Student's t-test of pairing design P values below 0.05 were considered significant

Pressure volume relationship of the isolated left atrium 16 male SHR and seventeen male WKR weighing 314±5 and 3_5±4 g respectively were compared in this study Their MAP values were 15?± mmHg and 106±2 mmHg respectively During ether anesthesia heart and lungs were excised and kept moist by ice-chilled Krebs solution during the preparation and the experimental procedure The right middle and the left pulmonary veins were cannulated (PE 50) while other veins were ligated close to the respective lung lobes To prevent communication between the left atrium and ventricle a tight ligature was placed around the heart just below the mitral valves. Left atrial pressure (LAP) was recorded via the catheter in the left pulmonary vein with the recording transducer at the level of the left atrium The isolated preparation thus consisting of the left atrium and adjacent parts of the pulmonary veins was expanded with saline stained with 2mM Cr EDTA, via the catheter placed in the right middle pulmonary vein The blood content was first rinsed out great care being taken to exclude air bubbles Prior to volume expansion the atrial preparation was allowed to empty passively against atmospheric pressure No collapse of the atrium was seen during this procedure From this level left atrial volume was increased stepwisely by adding 43 µl of saline via a step injector starting at 300 ms intervals For each step there was rapid (100–150 ms) increase of LAP which promptly levelled off when the 43 µl had been injected (Fig a b) In such a way LAP was increased in steps from the basal level of zero mmHg up to around 15 mmHg Thereafter the atrium was allowed to empty passively to again reach the basal level This procedure was repeated 5 times in each experiment and the pressure-

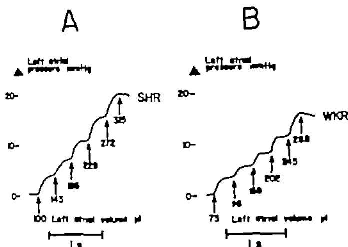


Fig 2 A typical experiment from SHR and WKR showing recording of atrial pressure and volume from isolated left atrium during rapid distension of the nonbeating atrium.

curves proved reproducible in all experiments. At the end of the experiment the absolute atrial volume was estimated the following way. From the basal level 86 μl of dyed solution was injected into the left atrium. This content was then washed out with ml of normal saline and the effluent was collected for spectrophotometrical determination of the Cr-EDTA concentration ([Cr EDTA]$_e$) and for volume measurement (V_e). The following equation was then used to estimate absolute left atrial volume:

$$\text{Left atrial volume} = \frac{[\text{Cr EDTA}]_e}{}\ V$$

The calculated value of the left atrial volume minus 86 μl was then the volume at the basal level where LAP was zero.

Three consecutive estimations of left atrial volume were performed in each experiment and the average coefficient of variation was 9–10%. In each experiment the relation between LAP and either absolute or relative volume changes was plotted and average pressure volume curves were constructed for each group. Distensibility of the left atrial wall was defined as percentage atrial volume increase per mmHg when increasing LAP from 2.5 mmHg to 12.5 mmHg. In all expts left atrial distensibility was determined by these rapid volume expansions and there called 'dynamic distensibility'. In 6–7 expts. of each group the left atrium was also distended slowly from the basal level by stepwise infusions of 39 μl during 25 s, LAP reaching 15 mmHg in about 3 min. So-called static distensibility was estimated from these values as described above. Also the percentage difference between the dynamic and static distensibility was calculated in each expt. The difference between SHR and WKR was compared using group comparison *t*-test. *P* values below 0.05 were considered as significant. In the text experimental results are given as mean ± S.E. unless otherwise stated.

RESULTS

Properties of isolated strips. The reference length of the strips in the two groups, i.e. the distance between the ligatures at a passive force of 4 mN was 2.8±0.16 mm for WKR and 3.3±0.18 for SHR respectively. The width of the strips as determined histologically was for WKR 0.548±0.029 mm and for SHR 0.560±0.036 mm. The cross-sectional areas of the dissected left atrial strips were 0.115±0.011 mm² for WKR and 0.117±0.012 mm² for SHR. Thus the dissection procedure yielded comparable tissue segments and there was no significant difference between WKR and SHR concerning the average wall thickness of the atrial strips, which was 0 11±0.019 mm and 0.207±0.012 mm respectively. The results of the studies on the relationship between the relative length increase of the strip (strain) and the passive force per cross-sectional area (stress) exerted by the successively extended strip are shown in Fig 1. Only at a length increase of 80% or more there is a significantly greater passive tension in the SHR tissue compared to WKR. Both in SHR and WKR the maximum active stress is reached at an elongation of the paced strip by 20–30%.

Pressure-volume relationship of the isolated left atrium. A typical pressure-volume recording of the rapidly distended, nonbeating isolated left atrium for SHR and WKR is shown in Fig 2. Such a recording showed very little variation at multiple runs in each expt. If there was a continuous de

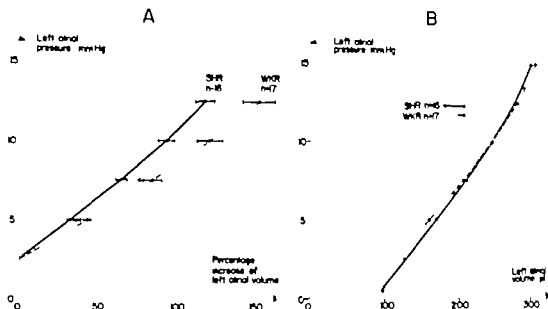


Fig 3a The average relationship between relative volume increase of left atrium and LAP in SHR and WKR during rapid distension of left atrium. The dynamic distensibility is significantly ($P<0.01$) decreased in SHR

Fig 3b The average relationship between absolute left atrial volume and LAP Error bars are omitted for clarity

velopment of rigor or oedema of the left atrial wall during the preparation and the experimental procedure a greater LAP at each left atrial volume would have developed with time The volume of the isolated left atrium at zero pressure tended to be greater in SHR compared to WKR 0 097±0 005 ml versus 0 079±0 007 ml ($P=0.053$) The relationship between LAP and relative as well as absolute volume changes of left atrium for the two groups is shown in Fig 3*a* *b* respectively A significantly greater relative increase of atrial volume is needed for WKR to achieve the same LAP as in SHR when rapidly increasing the LAP from 2 5 mmHg up to 12.5 mmHg The values for the dynamic distensibility were 11 7%±0 6/mmHg for SHR and 15 2%±1 2/mmHg for WKR ($P<0.01$) Thus the left atrial wall of SHR is about 23% less distensible than that of WKR when increasing LAP rapidly (0.5–1 s) from 2.5 mmHg to 12 5 mmHg

Fig 4 shows the average values of the pressure volume curves for both SHR and WKR from the experiments where the atrium was expanded both rapidly and slowly When distending the left atrium slowly (3 min) the distensibility of the atrium in creased markedly in both groups The static distensibility was 18.3±1.2%/mmHg for SHR and 19 1±2 1%/mmHg for WKR ($P=0.49$) The per cent difference between the dynamic and the static distensibility was significantly greater in SHR compared to WKR 29 8±3 9% versus 16.9±1.5% ($P=<0.01$)

DISCUSSION

The cardiac stretch receptors mainly located in the left atrial wall of the rat are activated during the rapid filling phase of the heart (Thorén et al 1978). A greater LAP is needed in SHR (~10 mmHg) to activate these receptors compared to normotensive controls (~5 mmHg) This resetting in SHR is either due to changes of the receptor itself or/and to a decreased distensibility of the left atrium i e a greater LAP is needed in SHR to cause the same distension of the left atrial wall The present study was designed to elucidate the properties of the left atrial wall since the properties of the stretch receptor itself cannot be studied directly

There was no difference in the thickness of the left atrial segments of SHR and WKR, when stretched by the same load These results do not indicate that the left atrial wall of SHR is increased in thickness although the distending LAP is twice as high compared to normotensive control rats (Noresson et al 1979) Greenberg et al (1978) recently showed signs of hypertrophy in the vascular walls of the caval vein the portal vein and the pulmonary artery in SHR compared to WKR However Mulvany & Ljung (1980) found no difference

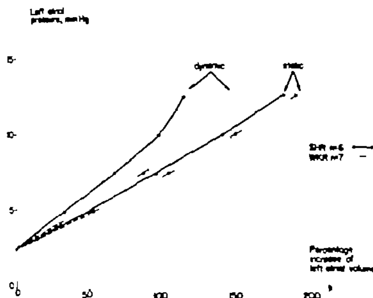


Fig. 4. Average pressure-volume curves from SHR and WKR where LAP is increased either slowly ('static distensibility') or rapidly ('dynamic distensibility') in the same experiment. There was no difference in static distensibility between the two groups. The percent difference between the dynamic and the static distensibility was significantly greater in SHR ($P<0.01$).

... wall thickness of the portal vein when comparing SHR and WKR.

The left atrial strip of SHR developed significantly greater passive tension per unit area compared to WKR only when elongated 80–100% from the reference length. The maximum active stress was reached at an extension of 20–30% from the reference length both in SHR and WKR. A further stretch resulted in a rapid decline in the active tension per unit area. Distension of the strip by 80–100% ... thus an unphysiologic overstretch. The greater passive tension at these exaggerated extensions in SHR can perhaps be explained by a greater content of collagen fibers per unit area of the strip compared to WKR since these fibres provide the increased stiffness at overstretch, at least in arteries (cf. Dobrin 1978).

Aars (1968) studied the elastic properties of aortic strips from renal hypertensive rabbits and found that they were thicker and less distensible per unit cross-sectional area than strips from normotensive rabbits. This decreased elasticity of aortic wall in renal hypertension can thus explain the resetting of the aortic baroreceptors shown by e.g. Aars (1968).

In the in vivo situation the cardiac stretch receptors are activated during the atrial v wave which lasts for 80–100 ms in the rat (Ricksten, unpublished observation). Thus, the atrial wall is distended very rapidly in vivo. Static load–length relationships of left atrial strips or aortic strips do not provide information about their in vivo elasticity when subjected to dynamic events such as the rapid filling phase of the heart and arterial pressure oscillations, respectively. It was therefore of particular interest to study the elastic properties of the left atrial wall in SHR during a rapid distension of the left atrium within a physiological pressure range. According to Fig. 2 the time for each injection of 43 μl saline (100–150 ms) is only slightly longer than the atrial v-wave. It was thus shown that the dynamic distensibility of the left atrium was significantly lower in SHR compared to WKR (Fig. 3*a*). There was, on the other hand, no significant difference in the static distensibility of the left atrium between the two groups. The decreased dynamic distensibility of the left atrial wall in SHR can in part explain the resetting of the cardiac receptors if it truly reflects distensibility also at the sites of main receptor location. In conscious rats LAP at end expiration is about 10 mmHg and 5 mmHg in SHR and nor

motensive control rats respectively (Noresson et al 1979) These values represent the threshold pressure for the left atrial receptor activation in the two groups (Thorén et al 1979) meaning that the receptors become activated in SHR and the normotensive control first at 10 mmHg and 5 mmHg respectively Assuming that there is no difference between the two groups concerning the characteristics of the receptor itself this means an equal degree of stretch for the SHR atrial receptors at 10 mmHg as for the WKR receptors at 5 mmHg In other words at the threshold pressure for receptor activation the absolute left atrial volume is equal in the two groups However in Fig 3*b* the absolute values for the left atrial volume is plotted aginst LAP for the two groups at rapid atrial distension If this dynamic pressure-volume curve truly mirrors the in vivo situation left atrial volume is about 50% greater in SHR at 10 mmHg compared to WKR at 5 mmHg Thus other factors than pure distensibility changes is presumably at hand in SHR in order to explain why the receptors start to fire at a LAP of 10 mmHg when left atrial volume is about 50% greater compared to normotensive controls

In conclusion the dynamic distensibility of left atrial wall is significantly decreased in SHR compared to WKR *This difference can at least partly* account for the resetting of the left atrial stretch receptors in SHR

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Stimulation of gastric acid and bicarbonate secretions by calcium in guinea pig stomach and amphibian isolated mucosa

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FLEMSTRÖM G. & GARNER, A Stimulation of gastric acid and bicarbonate secretions by calcium in guinea pig stomach and amphibian isolated mucosa. Acta Physiol Scand 1980 110: 419–426. Received 24 March 1980. ISSN 0001-6772. Department of Physiology and Medical Biophysics, University of Uppsala Biomedical Center Sweden.

Administration of Ca (1.5 mg/kg i.) increased the output of both H and HCO_3^- from the stomach of the anesthetized guinea pig as determined by measurement of gastric intraluminal pH and pCO_2. The rise in HCO_3^- secretion was slightly greater than that in H resulting in decrease in net acidity Fundic mucosa isolated from frogs was used to study the mechanisms of the stimulatory actions. An increase in Ca^{++} concentration in the nutrient (serosal) bathing solution from 1.8 to 7.2 mM stimulated H transport in this preparation. The effect of raising Ca^{++} concentration was inhibited by the histamine H_2 receptor antagonist Metiamide and by increasing nutrient Mg^{++} Stimulation of H transport, sensitive to Metiamide, was also observed with the calcium ionophore A23187 (4 µg/ml nutrient side). The results indicate that at the mucosal level, Ca stimulates H transport by release of histamine from mucosal stores with properties similar to those of mast cells. Transport of HCO_3^- in isolated mucosae was studied after inhibition of H^+ transport by metiamide. An increase in nutrient Ca^{++} concentration stimulated the HCO_3^- transport but the calcium ionophore had no effect. This action of Ca^{++} was abolished by atropine (10^{-6} M) and by raising nutrient Mg^{++} suggesting that it reflects release of acetylcholine from intramucosal nervous tissue. Thus Ca^{++} stimulated gastric transport of both H and HCO_3^- in vivo and in vitro but evidence for direct action on the transporting (parietal and epithelial) cells was not obtained.

Key words. Calcium, calcium ionophore, magnesium, gastric H secretion, gastric HCO_3^- secretion

Recent studies in vitro have shown that antral and fundic mucosae obtained from a variety of amphibia actively transport HCO_3^- into the gastric lumen (Flemström & Sachs 1975 Flemström 1976 1977) Secretion of HCO_3^- has also been demonstrated in the guinea pig stomach and dog fundic pouch in vivo (Garner & Flemström 1978 Kauffman, Reeve & Grossman 1980). It has been proposed that this HCO_3^- probably originating from surface epithelial cells, protects the mucosal surface from intraluminal acid (cf Flemström 1978). Inhibition of HCO_3^- transport by a variety of ulcerogenic agents, including acetazolamide, alpha-adrenergic agonists and nonsteroidal anti-inflammatory drugs as well as stimulation of secretion by gastric cytoprotective prostaglandins support this concept (Garner 1977 1978 Flemström 1981 Garner Flemström & Heylings 1979)

Gastric transport of HCO_3^- is usually smaller and thus masked by the simultaneous secretion of acid but can be revealed by specific inhibition of the latter (Flemström 1977). Previous studies on the effect of calcium ions on gastric electrolyte transport have considered the combined secretions of H and HCO_3^- i.e the net acid output. Depending on species and experimental conditions, administration of Ca^{++} has been found to either increase or decrease net acidity although the recorded effects are often relatively small (Barreras 1973) A Ca^{++} induced rise in HCO^- transport has been observed previously in isolated amphibian antrum (Flemström 1978) It was therefore of interest to compare the effects of Ca^{++} administration on gastric tran-

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sport of both H and HCO_3 It was found in the guinea pig stomach that a greater rise in HCO_3 transport masked a simultaneous rise in H transport Isolated fundic mucosae were used for further study of the stimulatory mechanisms

METHODS AND MATERIALS

In vivo experiments

A full description of the method has been published recently (Garner & Flemström 1978) Male albino guinea pigs (380–420 g) were starved for 24 h in cages with mesh bottoms to minimize coprophagy but allowed free access to drinking water Animals were anesthetised with urethane (1.5 g/kg intraperitoneally) Body temperature was maintained at 37°C with a heated blanket controlled by an intrarectal thermistor The abdomen was opened by a median epigastric incision and a multi-orifice rubber tube passed into the stomach through a cut in the duodenal wall proximal to the insertion of the bile duct The tube was secured in place by a ligature around the pylorus and exteriorised via the right flank The trachea was cannulated An umbilical artery catheter (8F Sherwood Medical Industries St Louis USA) was passed down the esophagus into the stomach so that the tip terminated just beyond the cardia The catheter was held in place by a tie around the esophagus but excluding the agil which also prevented contamination of gastric samples by salivary secretion The stomach was rinsed clear of food debris Then 10 ml volumes of mannitol solution (310 mM) were instilled into the stomach via the esophageal catheter and removed through the duodenal tube every 10 min

Intragastric pCO_2 was determined at the end of each 10 min instillation period by withdrawing samples from the stomach through glass capillary tubing into a pCO_2 electrode (Radiometer E5036) Intragastric pH was measured by a glass calomel electrode (Radiometer GK2321) Both electrodes were connected to expanded scale pH-mV meters (Radiometer pHM26)

Total H and HCO_3 in each instillate was calculated as described before (Garner & Flemström 1978) total HCO as free HCO (calculated from the pCO and pH values) *plus* dissolved CO_2 formed during reaction between secreted HCO and H Concentrations of Na and K in the gastric instillates were determined by flame photometry (Eppendorf Netheler and Hinz Hamburg) and Cl was determined by titration (Radiometer CMT 10)

In vitro experiments

The method has previously been described in detail (Flemström & Sachs 1975 Flemström 1977) Frogs (*Rana temporaria*) were purchased from Firma Panzer Lauingen–Donau FRG The animals were kept in tap water at 10°C for up to two months and force fed with ~200 mg of liver once a week The fundic mucosa (and submucosa) was separated from the serosa and muscle layers by blunt dissection in an unbuffered solution and mounted as a membrane between the two halves of an in vitro chamber The exposed mucosal area was 1 8 cm²

For comparison antral mucosa was used in some experiments. Antrum of sufficient size was obtained for *Necturus* or *Rana catesbeiana* and distinguished for fundus by its paler appearance

Each side of the mucosa was bathed with 20 ml c solution circulated by a gas lift An unbuffered solution gassed with 100% O prewashed in $Ba(OH)_2$ to exclude possible traces of CO_2, was used on the luminal (secretory) side The pH of this fluid was kept constant at 7 4 by infusion of iso-osmolar solutions containing 5 mM HCl or 10 mM NaOH under automatic control from a pH-stat system (Titrator TTT2 and Autoburette ABU 13 Radiometer Copenhagen) The amount of base or acid transported into the luminal solution was calculated from the amount of HCl or NaOH infused The nutrient (serosal) solution (pH 7.20) was buffered with HCO_3 (17.8 mM and phosphate (0 8 mM) and gassed with O_2 CO (95 5 vol./vol) The standard luminal and nutrient solutions contained (in mM) Na 102 4 K 4 0 Ca^{++} 1 8 Mg 0 8 and Cl 91 6 mannitol and SO^{--} being used on the luminal side to achieve iso-osmolarity (221 mosM) Open circuit transmucosal electrical potential difference was measured via matched calomel electrode and recorded on a high-input impedance voltmeter Electrical resistance was determined from the immediate (~1 sec) change in potential difference after sending current (30 $\mu A/cm^2$) through the mucosa in either direction Experiments were performed at 20°C

Drugs were administered on the nutrient side of the mucosa only Metiamide and calcium ionophore A2318 were kindly donated by Smith Kline and French Laboratories Welwyn Garden City England and El Lilly Sweden AB Stockholm Atropine (as sulphate) was obtained from Sigma St Louis, USA To examine the effect of raised Ca or Mg concentrations, the regular nutrient solution was replaced with solutions containing 7 2 mM Ca or Mg in which osmolarity was maintained by adjusting Na concentration Concentrations of Ca^{++} and Mg on the luminal side were always 1 8 and 0.8 mM respectively

Statistical analyse

Secretory rate and electrical properties in vitro were recorded every 5 min and mean values for consecutive 15 min periods calculated for individual experiments Overall mean ± S E of mean for each experimental group is presented Ion outputs in vivo were determined every 10 min Statistical significance in all experiments was determined using Student s t test for paired variates by comparing data during the 30 min period before exposure to Ca Mg or ionophore with 30 (or 15 min) data during and after administration

RESULTS

Stability conditions in controls

Mean rates of alkaline secretion in the guinea pig stomach in vivo and in Metiamide-treated fundus (*Rana temporaria*) in vitro are shown in Fig. 1 Secretory rate was stable in the guinea pig whereas

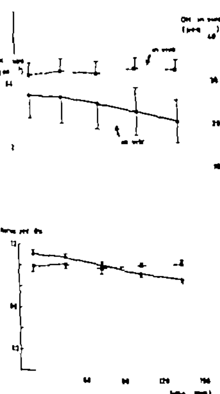


Fig. 1 Control rates of gastric alkaline secretion in frog isolated fundic mucosa and in guinea pig stomach in vivo. Mean rates of secretion during consecutive 30 min periods were calculated for each preparation. Overall mean ± S.E. at each time point is shown ($n=6$ for each group). Although there was considerable variation in secretory rate between individual preparations, intraexperimental variation was minimal as shown by the normalized data.

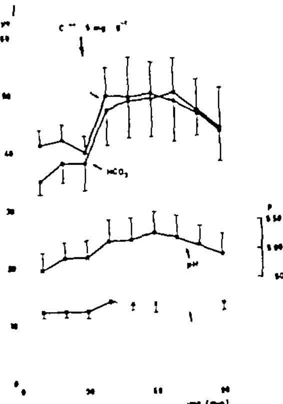


Fig. 2. In the guinea pig in vivo injection of Ca (1.5 mg/kg intravenously) stimulated secretion of both H and HCO_3 and also increased K output. Although peak H output occurred earlier HCO_3^- secretion rose by more than H secretion and rise in pH occurred. Mean ±S.E. is shown ($n=6$)

secretion in vitro showed slow but continuous decline. In both preparations, S.E. of the mean was large. However this was not due to variation within experiments but as the result of differences in secretory rate between individual preparations as shown by the normalised values. These were obtained by division of the rate in each 30 min period by the mean rate for all consecutive 30 min periods in the same experiment. Thus in studying the effects of ions or drugs on HCO^- transport, it was advantageous to use each experiment as its own control.

Gastric H and HCO transport in the guinea pig

The effects of i.v. injection of 1.5 mg/kg elemental Ca^{++} (as chloride) is shown in Fig. 2. Both HCO_3^- and H secretion increased significantly ($0.01<P<0.0?$ for HCO^- and $0.02<P<0.05$ for H) during the 30 min period after injection. Secretion of HCO_3^- was stimulated somewhat more than H as indicated also by the rise ($0.01<P<0.02$) in pH although peak H output appeared more rapidly in most of these expts. There was also a significant rise in K output ($0.02<P<0.05$). Output of Cl^- increased from 153±9 to 159±11 and Na from 134±15 to 141±11 μeq h but these changes were not significant ($P>0.05$).

The rise in HCO^- output in the guinea pig is in line with the previous finding (Flemström 1978) of stimulation of HCO^- transport by Ca in the isolated amphibian antrum. Relations between serum Ca levels, calcemic hormones and gastrointestinal function is, however complex (Cooper

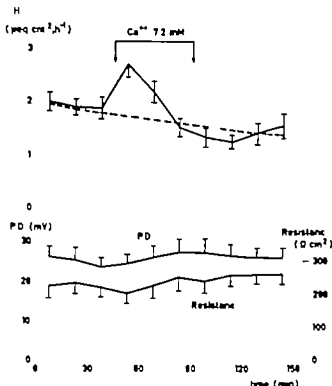


Fig 3 Effect of increasing Ca concentration in the nutrient (serosal) bathing fluid from 1 8 to 7.2 mM on titratable H secretion and electrical properties of isolated fundic mucosa Mean ± S E is shown ($n=10$) Spontaneous H secretion in an untreated preparation (dashed line) is also shown

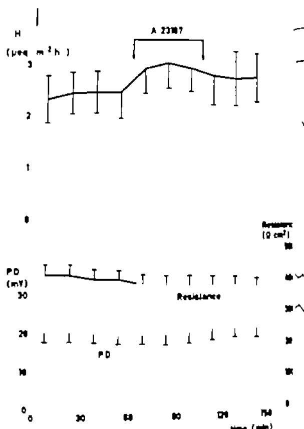


Fig 4 The calcium ionophore A23187 (4 µg/ml nutrient side) caused a significant increase in the rate of spontaneous net H secretion in isolated mucosa but did not affect the electrical potential difference or resistance Mean ± S E is shown ($n=8$)

et al 1978) Gastric H transport is under hormonal influence and evidence obtained in the dog suggests that this is also the case for gastric HCO transport (cf Allen & Garner 1980) Isolated mucosae were therefore chosen in the present study for further evaluation of the stimulatory actions of Ca or gastric ion transport Amphibian preparations were employed since it seems difficult to adequately oxygenate mammalian preparations at atmospheric oxygen pressure (Davenport & Jensen Chavré 1950 Flemström 1971)

H secreting isolated mucosa

Spontaneous (net H) secreting fundic mucosae were obtained from *Rana temporaria* Elevation of nutrient Ca from 1 8 to 7 2 mM increased H secretion ($P<0.01$) but the rise was of only 30 min duration (Fig 3) Secretion then decreased to values below those expected from the spontaneous decline occurring in untreated mucosae and after return to 1 8 mM nutrient side Ca there was some recovery in secretory rate There was thus a two-phase response to raising Ca concentration an increase in net H secretory rate followed by a decrease Only minor changes in the electrical potential difference and resistance occurred during these expts

The calcium ionophore A23187 (4 µg ml⁻¹) increased the rate of (net) H secretion ($P<0.01$) but produced no changes in the electrical parameters of the mucosa (Fig 4) A lower concentration of the ionophore (0 8 µg ml $n=6$) had no significant effects The ionophore was added as a small volume from an ethanol-containing stock solution but the ethanol alone (0 4% vol./vol nutrient side) did not affect H secretion ($n=7$)

Mg antagonizes stimulation of gastric (net) H secretion by intravenous or intragastric Ca in man (Deyhle Miederer & Ottenjahn 1970 Christiansen Rehfeld & Kirkegaard 1979) Elevation of Mg concentration in the nutrient solution from 0 8 to 7 2 mM (Fig 5) depressed spontaneous H secretion and markedly reduced the stimulatory effect of Ca (cf Fig 3 and 5)

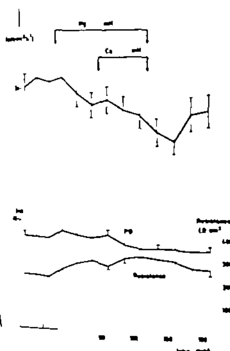


Fig 5 In spontaneous (net H^+) secreting isolated mucosa, nutrient side Mg^{++} and Ca^{++} concentrations were increased from 1 8 to 7 mM as indicated. Mg^{++} inhibited secretion and the stimulatory effect of raised Ca^{++} Mean ±SE shown (7)

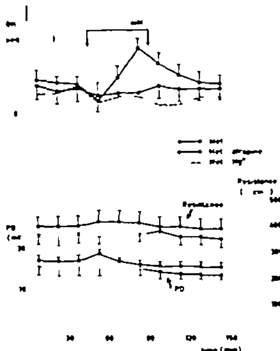


Fig 6 Fundic mucosa was pretreated with Metiamide (10^{-4} M nutrient side) until net acid secretion had ceased and a steady rate of net alkaline secretion appeared. This drug was then present throughout the experiments. An increase in nutrient Ca^{++} from 1.8 to 7.2 mM caused slight initial decrease in secretory rate followed by an increase to level above the starting rate (=12). Ca had no effect when atropine (10^{-4} M nutrient side) was present (=9) or when nutrient Mg^{++} had been increased from 0 8 to 7.2 mM (n=8, dashed line).

HCO_3^- secreting isolated mucosa

Fundic mucosae were pretreated with the histamine H_2 receptor antagonist Metiamide (10^{-4} M nutrient side) and, after net H secretion had ceased and a steady rate of titratable alkalinization had appeared, nutrient Ca^{++} was raised to 7.2 mM (Fig. 6) Secretory rate was slightly but not significantly (P>0 05) lower during the first 15 min period but then increased and was significantly (P<0 01) higher than the control rate during the final 15 min period of exposure to high Ca There was an initial rise in the transepithelial electrical potential difference (0.01<P<0 02) but no significant change in the electrical resistance

Fig 6 also illustrates the effects of treatment with atropine (10^{-4} M) or a high Mg^{++} (7.2 mM) concentration These conditions per se did not affect the rate of alkaline secretion or the electrical parameters (n=8 for both) but abolished the stimulatory effect of increased Ca^{++}

When calcium ionophore A23187 was added to Metiamide treated alkaline secreting mucosae there was no change in the secretory rate even at a concentration of 10 μg, ml (Fig. 7) Stimulation of H transport (cf Fig. 4) would have resulted in a decline in titratable alkalinization and stimulation of HCO^- transport in an increase To exclude the possibility that stimulation of equal magnitude in the rates of H and HCO_3^- secretion occurs some experiments were performed with spontaneously alkalinizing antral mucosae No effects with the ionophore (4 μg, ml^-) were observed in antrum from either *Rana cat belana* (=4) or *Necturus* (n=5)

DISCUSSION

Studies in vitro and in vivo have demonstrated that gastric mucosa transports base as well as acid into the lumen of the stomach and that the pre

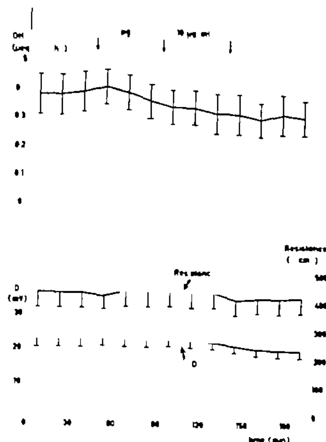


Fig 7 In isolated fundic mucosa pretreated with Metiamide (10^{-4} M nutrient side) the calcium ionophore *A23187 had no effect on alkaline secretion or electrical* properties There was a slight decline in secretory rate but this was similar to controls (see Fig. 1) Mean ± S E is shown ($n = 10$)

vailing net acidity of this combined secretion is the difference between the larger output of H ions and the smaller and therefore masked output of HCO_3 (Flemström 1977 Garner & Flemstrom 1978 Kauffman et al 1980) It is well established that H ions are secreted by parietal cells in the fundic area while in vitro studies indicate that HCO_3 very probably originates from surface epithelial cells in both fundus and antrum (Flemström 1977)

Previous work on the effect of Ca on gastric secretion of electrolytes has considered effects on the combined secretions of H and HCO Administration of Ca has been reported to decrease net acid secretion in the dog and rat in vivo (Barreras 1973) and in the amphibian gastric mucosa in vitro (Kasbekar & Chugani 1976) In other species including man ferret cat and monkey net H secretion is increased by intravenous or intragastric Ca (Barreras 197 Christiansen et al 1979) The decrease in (ne acid output observed in some species in vivo m only be apparent and reflect greater stimulation of HCO_3 transport as observed in the preser study in the guinea pig (Fig 2) Raising nutrien Ca concentration increased net H secretion i vitro (Fig 3) but this effect was transient a followed by a decline in titratable acidity T decline in view of results in the isolated antr (Flemström 1978) and in Metiamide treated fun mucosa (Fig 6) is probably due to an increase HCO transport rather than actual inhibition H transport

Calcium is important in maintaining norm permeability characteristics of most epithelia a removal of this ion increases paracellular perme tion of ions across the gastric mucosa (Forte Nauss 1963 Sedar & Forte 1964 Chung et al 1970 However transmucosal electrical resistance nev decreased as a result of the alterations in nutrie Ca employed in the present study It is thus u likely that any increase in mucosal permeability t HCO_3 (or H) occurred Passive permeation c HCO_3 contributes to luminal alkalinization in a trum (Flemström & Sachs 1975) but not in fundu (Flemström 1977) An increase in paracellular per meation of ions masked by a concomitant decreas of equal magnitude in transcellular ion permeabilit cannot be excluded but would seem unlikely

Several mechanisms have been suggested t account for the stimulation of (net) H secretion b Ca observed in some species In man gastri released from the antrum or extragastric source contributes to the rise in acid output after ad ministration of Ca (Barreras 1973 Christian sen et al 1979) However Mg antagonises the effect of Ca on H secretion in man and under some conditions intragastric Ca increases H secretion but not serum gastrin levels (Holtermuller et al 1974) Thus direct stimulation of the parietal cells by Ca or its sensitization to other stimuli has also been postulated

In the present study administration of Ca i increased H secretion in vivo and in the fundus in vitro The latter preparation would seem to exclude an effect mediated by release of gastrin Administration of the calcium ionophore A23187 also increased (net) H secretion in vitro In alkaline secreting mucosae treated with the histamine H_2 receptor antagonist Metiamide any stimulation of

H⁺ transport by Ca and A_3187 comparable in size to that occurring in untreated mucosae would have resulted in a sharp decline in titratable alkalinity This as never observed (Figs. 6 and 7), suggesting that stimulation of H secretion by both Ca⁺⁺ and calcium ionophore is mediated by histamine. As in man (Deyhle et al. 1970 Christiansen et al. 1979), Mg⁺⁺ depressed both spontaneous H⁺ secretion and the stimulatory effect of increased Ca⁺⁺ Competition between Ca and Mg in the release of histamine has been demonstrated in isolated mast cells (Foreman Mongar & Gomperts 1973) and these cells are proposed to be the major repository for gastric mucosal histamine (Soll Lewin & Beaven 1979) Furthermore spontaneous H secretion in isolated mucosae is probably accounted for by histamine released from endogenous sources (Kasbekar 1967 Rangachari 1975)

Previous work has demonstrated that gastric HCO_3^- transport is stimulated by carbachol dibutyryl-cGMP and ulceroprotective prostaglandins while histamine and gastrin are without effect. Atropine prevents *stimulation* of HCO_3 secretion by carbachol in the guinea pig but does not affect basal (unstimulated) HCO_3^- transport (Garner & Flemström 1978) It was found in the present study that atropine is also without effect on basal HCO_3^- transport in vitro An increase in luminal Ca⁺⁺ concentration but not administration of calcium ionophore stimulated HCO_3 transport. The effect of Ca⁺⁺ was abolished by pretreatment with either atropine or increased Mg concentration. These results suggest that Ca induced stimulation of HCO_3^- transport is mediated by acetylcholine Release of acetylcholine from nervous tissue in isolated ileal preparations depends on external Ca⁺⁺ and is reduced by external Mg (Paton & Vizi 1968, Paton, Vizi & Car 1971) Evidence for a direct effect of Ca ions on the HCO_3^- transporting cell was not obtained in the present study Thus Ca stimulates gastric transport of both H⁺ and HCO_3^- but effects at the mucosal level are probably mediated by release of histamine and acetylcholine respectively

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Factor in human CSF with apparent morphine-antagonistic properties

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Chronic use of opiates leads to development of tolerance. This is a very characteristic phenomenon. Two mechanisms that could explain development of tolerance are 1) that the receptor changes in responsiveness on chronic exposure or 2) that counteracting neuronal or endocrine systems "anti-opioids" are activated. The latter possibility has been investigated by us (Terenius 1975), Ungar and co-workers (Ungar & Ungar 1976, Ungar et al. 1977) and more recently by Han and co-workers (Han et al. 1979). All these investigators obtained evidence for anti-opioid substances believed to act by competition at the level of opiate receptors. However the studied fractions were not well defined chemically and the results are not easy to interpret.

We previously used the human cerebrospinal fluid (CSF) as a source of neuroactive substances, particularly endorphins. We now report on observations in relation to the search for antiopioid substances in CSF of patients, particularly of narcotic addicts.

The lumbar CSF samples were obtained from heroin addicts who had been admitted to hospital for detoxification and start on methadone maintenance therapy. Control CSF was obtained from patients undergoing neurosurgery but who had no history of psychiatric or neurologic disturbances. All samples of CSF were kept frozen at −90°C until analyzed. After thawing they were desalted on a SP Sephadex ion-exchanger (15×1 cm) and active material was eluted in 0.5 M pyridine acetate buffer. Further fractionation was accomplished using a Biogel P2 column (160×1 cm) eluted in 0.1% trifluoroacetic acid. This column had previously been calibrated with peptides of known molecular weight. Details of the procedure are published elsewhere (Wahlström & Terenius 1980). Fractions were lyophilized and tested for activity using either the electrically stimulated myenteric plexus-longitudinal muscle preparation of guinea-pig ileum or the receptor binding assay. When the guinea-pig ileum assay (Paton 1957) was employed fractions were tested either alone or in the presence of normorphine at a dose which reduced the twitch approximately by half (about 10^{-} M concentration). The receptor binding assay followed a previously described procedure (Terenius 1974). D-Ala^2,Leu-enkephalin-H was the radioligand and the fractions were added in graded amounts. Activity was read against a standard curve for D-Ala^2 Leu^5-enkephalin.

Fractionation of material on the Biogel P2 column resolved several components active on the ileum preparation. One fraction with apparent molecular weight, 1000–1500 dalton, suppressed the twitch, i.e. mimicked the action of normorphine. Another fraction of 1500–3000 dalton molecular weight counteracted normorphine (Fig. 1a) whereas in the

Table 1 *Relative amounts of a fraction from human CSF potentiating the twitch of the electrically stimulated isolated guinea-pig ileum*

Origin of CSF	Pool	Concentration A.U./10 ml CSF*
Heroin addicts in withdrawal	1	1.3
	2	0.6
Non-addicts	1	0.1
	2	0.1
	3	0.2

* 1 A.U. will increase the electrically induced twitch of the preparation by 30%

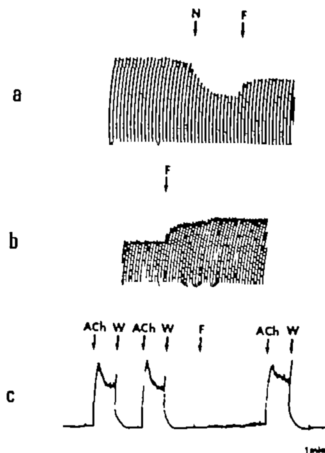


Fig. 1. Guinea-pig ileum longitudinal muscle preparation in Krebs-bicarbonate solution. In *a* and *b* it was stimulated electrically at a frequency of 0.1 Hz. The bath volume was 2.7 ml. (*a*) Recording of the reversal of the normorphine-induced suppression of the twitch. Normophine (N) concentration was ×10⁻ M. The antagonistic factor (F) originates from 100 ml control CSF. (*b*) Increase of the magnitude of the contraction by the factor (F) from 200 ml control CSF. (*c*) No effect of factor (F) from 200 ml of control CSF on the contraction produced by 3 μg acetylcholine (ACh). (W) = wash.

absence of normorphine this fraction potentiated the twitch (Fig. 1*b*). The potentiation was not influenced by naloxone but was completely blocked by atropine.

The possibility of interaction between the antagonistic factor and exogenously applied acetylcholine was tested in a separate experiment. Acetylcholine at a dose of 3μg caused about the same contraction as that produced by the electric pulse. In the presence of the fraction there was no change in response (Fig. 1*c*). The same fraction showed weak affinity in a receptor assay with D-Ala[2] Leu[5]-enkephalin-H as radio-indicator: the amount of this fraction which had increased the twitch two-fold inhibited binding by 33 %. The fraction contained no material which was recognized by substance P-directed antibodies.

As illustrated in Table 1, particularly high concentrations of the antagonistic factor were found in CSF from narcotic addicts during abstinence.

The data presented here confirm the existence of a factor with apparent anti-opioid activity. However, since the factor described here also potentiates the electrically-induced contraction in a morphine-naive ileum preparation, it is not a pharmacologic antagonist. That is to say, it does not appear to compete with opioids for their receptors as naloxone does. By itself naloxone does not affect electrically induced contractions of guinea-pig ileum. Morphine inhibits contraction of the ileum myenteric plexus preparation which is thought to be a consequence of its ability to block release of acetylcholine (Paton 1957, Lees et al. 1972). The CSF factor may have the opposite effect, i.e. stimulating the release, although direct evidence is lacking at present.

The relation of the present antagonistic factor to the material isolated from brain and described by others to be "antiopioid" (Ungar & Ungar 197-, Han et al. 1979) is unclear since neither of these groups tested their material on a morphine-naive preparation and because chemical identification is lacking. The elevated concentrations of the present factor in CSF from addicts indicates its potential functional significance.

This work was supported by the National Institute on Drug Abuse (USA) and the Swedish Medical Research Council. Dr Bengt Sjölund, University of Lund, Sweden, kindly provided control CSF samples.

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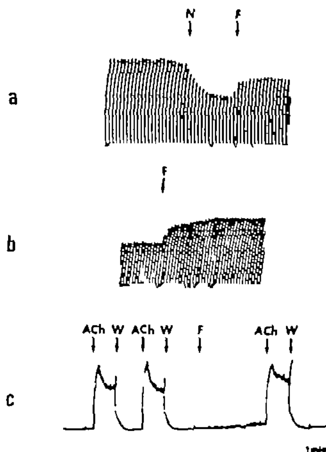


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Characteristics of right ventricular receptors with non-medullated vagal afferents in the cat

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There is a large population of left ventricular receptors signalling in non-medullated vagal afferents in dogs (Coleridge et al. 1964 Muers & Sleight 1972) and cats. When activated, these receptors induce powerful depressor reflexes, characterized by bradycardia and vasodilatation (Thorén 1979). These receptors are also the main receptor stations for eliciting the Bezold-Jarisch reflex (Thorén 1979). The receptor discharge in relation to changes in cardiac dynamics was recently described by Thorén (1977) and the receptors seemed to respond mainly to changes in left ventricular end-diastolic pressure but not the isolated changes in ventricular systolic pressure. There also exist a sparse population of receptors with non-medullated afferents in the right ventricle of the dog (Coleridge et al. 1964 Muers & Sleight 1972). However these authors did not describe the firing characteristics of the receptors. The aim of the present study therefore was to examine the firing characteristics of right ventricular C-fibres with non-medullated vagal afferents.

Cats, anesthetized with chloralose were thoracotomized, the pericardium was opened and snares placed around the ascending aorta and the pulmonary artery. The right vagus was cut below the entrance of the cardiac nerve. This eliminated almost completely the afferent traffic from the pulmonary inflation receptors, which greatly facilitated the identification of and recording from, cardiac afferents.

Pressures were measured via catheters inserted into the aorta and right atrium and connected to Statham 23DC transducers. The pressure in the right ventricle was measured via a thin needle inserted into the ventricle.

Thin filaments from the right cervical vagus were placed on an electrode for recordings of impulse activity as described previously by Thorén (1977). Conduction velocity was measured by application of an electrical stimulus to the right cardiac nerve and recording the evoked potential in the neck. As a screening procedure to find right ventricular C-fibres which normally have a low irregular discharge the activity in all filaments dissected from the vagus was observed during a brief occlusion of the pulmonary artery. Localization of the receptors to the right ventricle was further established by the failure of the receptors to increase their firing during a balloon occlusion of the bicuspid valve and by probing of the heart with fine plastic probes.

The study is based on recordings from 8 receptors in 7 cats. The endings were located throughout the entire right ventricle. The conduction velocity in the cervical vagus was 0.8±0.1 m/s (mean ± S.E.) and the total conduction time from the receptor site to the recording electrode was from 115 to 250 ms. Two of the receptors had spontaneous activity during normal conditions, one ending in phase with the cardiac cycle and one ending with a low frequent irregular activity. The other endings were silent.

The receptor response to graded pulmonary artery occlusion was also examined. Upon activation 3 endings showed mainly irregular discharge with no obvious relation to the cardiac cycle but the other 5 receptors discharged with cardiac rythmicity. When taking the total conduction time into account all these endings were activated in systole just as the endings in the left ventricle. The relation between the receptor activity and changes in right ventricular systolic and end-diastolic pressure is shown in Fig. 1. Upon a graded increase in right ventricular afterload there is a simultaneous increase in both systolic and end-diastolic pressure and a parallel increase in receptor discharge.

pressure, despite the fact that activation occurred during systole. The reason for this paradoxical finding is probably that changes in filling pressure of the left ventricle ill markedly influence the following systole by the Starling mechanism as discussed by Thorén (1979). The reason why isolated changes in systolic pressure do not influence the receptor activity is probably that the left ventricle can stand small or moderate changes in afterload without changing the end-diastolic pressure due to the so-called homeometric autoregulation.

Right ventricular receptors are also activated during systole indicating that the ystolic contraction is of major importance for the receptor activation. However the activity seems to increase parallel with the changes in both right entricular systolic and end-diastolic pressure. The reason is probably that the right ventricle does not show any evident homeometric autoregulation, meaning that even a fairly small or moderate change in the right entricular systolic pressure will induce a clear rise in the right ventricular end-diastolic pressure.

Thus the conclusion from this study is that the characteristics of all the mechanosensitive C-fibre endings in the heart are ery much dependent on the dynamics of the myocardium in which the receptors are situated. Thus, left ventricular C-fibre endings do not change their activity upon moderate changes in left ventricular systolic pressure (Thorén 1979) but are fairly sensitive to changes in left ventricular end-diastolic pressure due to the fact that the left ventricle can stand small or moderate changes in systolic pressure without changing end-diastolic volume. In contrast C-fibres in the atria respond mainly to the atrial distension during the ..., simply because the receptor endings are maximally deformed during the atrial filling. The right entricular C-fibre endings are activated during the right ventricular systole because the receptor site is then maximally deformed. Due to the dynamics of the right entricle the activity of the receptors correlates both with changes in right ventricular systolic and end-diastolic pressure.

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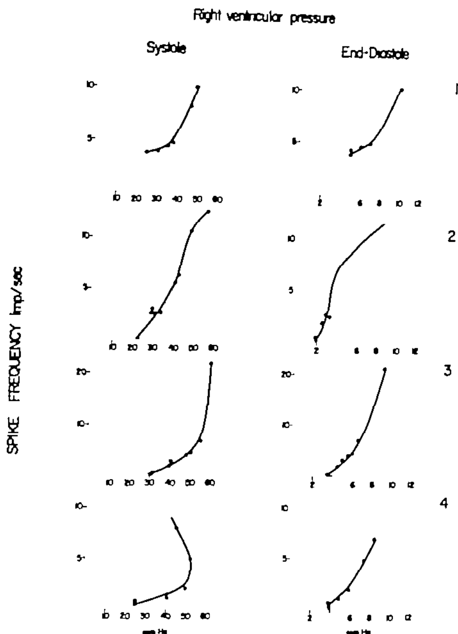


Fig 1 Pressure discharge curves for 4 right ventricular C-fibre endings during graded pulmonary artery occlusion The activity for each receptor is plotted against the right ventricular systolic and end-diastolic pressure

The present study describes C fibre endings determined on basis of conduction velocity measurements and originating in the right ventricle of the cat. However in relation to the large number of receptors earlier found in the left ventricle (Thorén 1977 1979) the population of right ventricular C fibres seems to be small and this is also likely to be the reason why distension of the right ventricle induces only weak circulatory reflexes (Aviado et al 1951 Barer & Kottagoda 1958 Ross et al 1961) The relatively small muscle mass of the right in comparison with the left ventricle might be the reason for the small numbers of endings

When activated the right ventricular receptors as well as the left ventricular receptors display a cardiac rhythmicity in systole indicating that the systolic contraction of the ventricles is of major importance for the receptor activation During aortic occlusion or transfusion the activity in the left ventricular C fibre endings correlated well with changes in left ventricular end-diastolic pressure but not with changes in the left ventricular systolic

pressure despite the fact that activation occurred during systole. The reason for this paradoxal finding is probably that changes in filling pressure of the left ventricle will markedly influence the following systole by the Starling mechanism as discussed by Thorén (1979). The reason why isolate changes in systolic pressure do not influence the receptor activity is probably that the left ventricle can stand small or moderate changes in afterload without changing the end-diastolic pressure due to the so-called homeometric autoregulation.

Right ventricular receptors are also activated during systole indicating that the systolic contraction is of major importance for the receptor activation. However the activity seems to increase parallel with the changes in both right ventricular systolic and end-diastolic pressure. The reason is probably that the right ventricle does not show any evident homeometric autoregulation meaning that even a fairly small or moderate change in the right ventricular systolic pressure will induce a clear rise in the right ventricular end-diastolic pressure.

Thus the conclusion from this study is that the characteristics of all the mechanosensitive C-fibre endings in the heart are very much dependent on the dynamics of the myocardium in which the receptors are situated. Thus, left ventricular C-fibre endings do not change their activity upon moderate changes in left ventricular systolic pressure (Thorén 1979) but are fairly sensitive to changes in left ventricular end-diastolic pressure, due to the fact that the left ventricle can stand small or moderate changes in systolic pressure without changing end-diastolic volume. In contrast C-fibres in the atria respond mainly to the atrial distension during the v-wave, simply because the receptor endings are maximally deformed during the atrial filling. The right ventricular C-fibre endings are activated during the right ventricular systole because the receptor site is then maximally deformed. Due to the dynamics of the right ventricle the activity of the receptors correlates both with changes in right ventricular systolic and end-diastolic pressure.

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Excessive cellular acidosis an important mechanism of neuronal damage in the brain?

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There is evidence that in cerebral hypoxia/ischemia of sufficient severity (and duration) to induce neuronal cell damage, cellular energy metabolism is grossly perturbed However some results suggest that the damage incurred does not always correlate to the energy failure For example seemingly paradoxical results were obtained when metabolic recovery was studied after 30 min of either complete, or severe incomplete ischemia (cerebral blood flow zero and ≤5 % of control respectively) In these experiments, in spite of a similar perturbation of cerebral energy state during ischemia, metabolic recovery was clearly better following complete ischemia (Nordström et al 1978 see also Hossmann & Kleihues 1973) It was tempting to conclude that, in incomplete ischemia, the scant supply of oxygen or substrate was responsible for the serious outcome. So far attempts to prove the existence of oxidative breakdown of tissue constituents, e g through lipid peroxidation, have failed (Rehncrona et al 1980 b), and recent interest has been focussed on the consequences of a continued glucose supply to the ischemic tissue.

In a previous study from this laboratory an attempt was made to study the influence of acidosis on recovery following complete ischemia (Ljunggren et al 1974). In this condition, the resulting lactic acidosis is determined by the preischemic stores of glucose and glycogen which were varied by insulin injection (hypoglycemia) or glucose administration (hyperglycemia) In spite of the fact that, at the end of 5 min of ischemia, tissue lactic acid concentrations varied between about 5 and about 20 μmol g metabolic recovery was similar

In a recent series of expts. Myers and associates have reported that with more prolonged periods of hypoxia and ischemia, recovery varies with the nutritional state of the animals, and the results suggest that tissue lactic acid levels exceeding about 20 μmol g is detrimental for recovery (for literature see Myers 1979)

The present expts. were designed to study the influence of blood (and tissue) glucose levels on recovery following severe incomplete ischemia of 30 min duration. In addition, tissue glucose levels were similarly manipulated during complete ischemia.

Method The experiments were performed on male Wistar rats (250–350 g) that were either allowed free access to rat pellets and tap water or starved for 16–24 h prior to expts After induction of anesthesia with halothane and operation under 0.6% halothane and 70% N_2O the animals were maintained artificially ventilated on 70% N_2O and 30% O_2, their body temperature being maintained at 37°C. Physiological parameters measured included blood pressure pH and blood gases, as well as EEG and somatosensory evoked potentials

Complete ischemia was induced by raising the CSF pressure above the systolic blood pressure by infusion of an artificial CSF solution into the cisterna magna, and incomplete ischemia by ligation of the carotid arteries combined with a lowering of mean arterial blood pressure to 50 mmHg (for details, see Nordström et al. 1978, Rehncrona et al 1980 b) The period of ischemia was 30 min and recovery of 90 min duration was induced by discontinuation of CSF infusion and removal of carotid clamps with reinfusion of blood, respectively Tissue was frozen for metabolic analyses at the end of ischemia and at the end of recovery (separate groups)

The following groups of animals exposed to complete ischemia were studied. ( ) normally fed animals, (*b*) fasted animals infused i with 2 ml of 50% glucose solution during 15 min prior to induction of ischemia and ( ) fasted animals infused

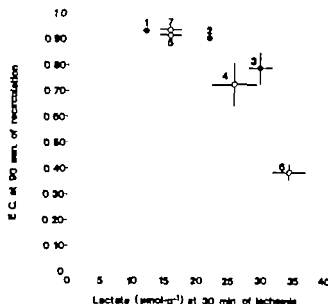


Fig 1 The adenylate energy charge

$$E.C = \frac{[ATP]+0.5[ADP]}{[ATP]+[ADP]+[AMP]}$$

of rat cortical tissue at 90 min of recirculation following 30 min of cerebral ischemia as a function of the cortical lactate concentration at the end of ischemia. Experimental groups. ■ Non-ischemic controls ● reversible complete ischemia. 1 normally fed fasted infused with glucose prior to ischemia 3 fasted hypercapnic and infused with glucose prior to ischemia. O Reversible incomplete ischemia. *4 normally fed 5 fasted infused with* Krebs Hensleit solution prior to ischemia 6 fasted infused with glucose prior to ischemia, 7 fasted infused with glucose during recirculation There were 6 or 8 animals in each group The symbols represent mean ± S.E Postischemic E C. differed significantly ($P<0.001$ Student s *t*-test) from controls in groups with ischemic lactate concentrations exceeding 20 μmol g

similarly after Pa_{CO_2} had been increased to 100–120 mmHg Hypercapnia was induced to further increase tissue glucose concentrations The groups exposed to severe incomplete ischemia were as follows (*a*) fed animals (*b*) fasted animals infused with 2 ml Krebs Hensleit solution (*c*) fasted animals infused with 2 ml of a 50% glucose solution and (*d*) fasted animals given an equal amount of glucose at the end of the ischemic period The latter group was included to exclude the possibility that an elevated glucose concentration during recirculation affected metabolic recovery

Results and Discussion In all groups studied pre- and postischemic physiological parameters were similar and in all animals spontaneous or induced electrical activity ceased during ischemia In all animals deterioration of cerebral energy state during ischemia as evaluated from the concentration of PCr ATP ADP and AMP was similar Preischemic glucose concentrations varied between about 5 μmol g⁻¹ (fasted animals) and 35 μmol g⁻¹ (glucose-infused animals)

The main results are illustrated in Fig. 1 which relates the calculated energy charge of the adenine nucleotide pool at the end of the 90 min recirculation period to the tissue lactic acid concentration at the end of the 30 min period of ischemia. The following points emerge (1) Following complete ischemia metabolic recovery is adversely affected if tissue lactic acid concentrations during ischemia are raised by glucose infusion at normal and increased CO_2 tension (2) If animals are fasted recovery is at least as good following severe incomplete as following complete ischemia. In fact in the fasted animals extensive recovery of EEG and evoked potentials was observed (3) Glucose infusion instituted before (but not after) the period of incomplete ischemia adversely affects recovery (4) A detrimental effect of lactic acid is noted if its concentration exceeds 20–25 μmol g

In summary our results suggest that if the tissue hypoxia is sufficiently severe to disrupt the phosphorylation state of the tissue excessive lactic acidosis is detrimental to recovery The results offer an explanation for the paradoxical results reported in the introduction and also seem to explain why other authors have failed to find that incomplete ischemia is more harmful than complete ischemia (Steen et al 1979) Thus in these experiments fasted animals were used and the period of ischemia was limited to 10 min However the mechanisms whereby acidosis disrupts cerebral metabolism remain to be defined

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Potential clamp of demyelinated rat nerve fibres

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Segmental demyelination was induced in the sciatic nerve of rats by intraneural injection of diphtheria toxin (Allt & Cavanagh 1969) Rats (300–400 g Sprague-Dawley) were anesthetized and given 0.5 Lf diphtheria toxin in 5 µl buffered sterile isotonic NaCl solution by microinjection into the exposed sciatic nerve. About 14 days later the rats were sacrificed and the nerve was investigated Pronounced segmental demyelination was seen in the fibres at the site of injection. A large diameter (> 10 µm) fibre with only paranodal demyelination was selected and dissected free from the rest over the neighbouring internodes. The fibre was photographed and then mounted in the recording chamber for the potential clamp analysis (Brismar 1980)

Fig 1 shows fibre with paranodal demyelination obtained in this way The membrane currents of this fibre are shown in Fig Positive potential steps were associated with comparatively small inward currents and the reversal potential for the initial current was low (about 0 mV) The delayed outward currents and the inward current tails were considerably larger than in normal fibres.

The ionic specificity of the membrane currents was tested by exchange of the external Ringer solution to a solution with high [K]. In this solution the initial inward current was negligible and the delayed currents became inward at potentials below −10 mV and outward at more positive potentials The delayed currents were blocked in 5 mM TEA (tetra etyl ammonium chloride)

The membrane leak conductance (measured from the current associated with negative potential steps and calculated for an assumed nodal width of 1 0 µm .e for an unchanged nodal surface area) was 150 mS cm that is similar to that in normal fibres. The membrane time constant (measured from the recorded membrane potential change associated



Fig 1 Paranodal demyelination in rat nerve fibre Photomicrograph (LEITZ inverted microscope dark field illumination) of fibre from sciatic nerve 14 days after diphtheria toxin injection Arrows show demyelinated d regions.

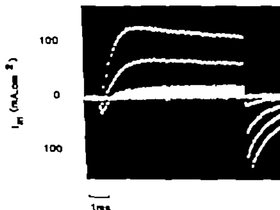


Fig 2 Membrane currents (I_m) of potential clamped demyelinated rat nerve fibre (shown in Fig 1) Holding potential −80 mV positive potential pulses to 60, 40 and −20 mV respectively associated traces of membrane current superimposed in the figure. External Ringer solution 147 mM-NaCl, 5 9 mM KCl, 3 1 mM-$CaCl_2$, 5 0 mM Tris-SIGMA pH 7 4 Current calibration tentatively performed as for normal fibres, for a nodal gap width 1.0 µm (Brismar 1980) Temp. 24°C.

with a current pulse) was 0 55 ms which was almost 10 times longer than normally suggesting an increase in the nodal capacitance

It is concluded that paranodal demyelination was associated with an increase in the axoplasmic [Na] and an increase in the delayed K-permeability which normally is small in rat fibres (Brismar 1980) A similar increase in the delayed currents was described in fibres of rats with alloxan induced diabetes (Brismar 1979) There is further evidence of an increase in nodal capacitance related to the demyelination whereas the nodal resistance at resting potential was little affected

Summary Potential clamp analysis of paranodally demyelinated nerve fibres (induced by diphtheria toxin) showed decreased inward Na-currents considerably increased delayed K-permeability and i creased nodal membrane capacitance

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Autoinhibition versus transsynaptic control of noradrenaline secretion in the rabbit kidney

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Ample experimental evidence indicates the presence of presynaptic α-adrenoceptors, through which the neurotransmitter noradrenaline (NA) is believed to restrict its own release (cf. Langer 1979). In addition to these NA autoreceptors, there seems to be other presynaptic receptors which mediate feedback control of NA release by prostaglandins and purines, substances preferredly derived from postjunctional elements (cf. Hedqvist 1977, Fredholm & Hedqvist 1980). In this communication data are presented, which indicate that transsynaptic feedback substantially contributes to the control of NA secretion, being at least as effective as NA autoregulation, in the rabbit kidney.

Methods. Rabbits of either sex, weighing 1.5–2.5 kg were anesthetized with pentobarbital and the left kidney with its nervous and vascular supply was isolated and perfused at a constant rate of 10 ml/min with a slightly modified Tyrode's solution, as previously described (Hedqvist, Fredholm & Ölundh 1978). The experiment was started with a 15 min intraarterial infusion of ^{3}H-(−)-NA (25 μCi, 40 μCi/mmol; Amersham) or ^{14}C-adenine (5 μCi, 296 μCi/mmol; Amersham) followed by a 30 min washout period. Thereafter the renal nerves were stimulated by means of platinum electrodes and a Grass S4 stimulator delivering trains of pulses (3 Hz, 1 ms, 10 V) at intervals of usually 10 min. The perfusate was collected in 1 min fractions and the radioactivity was determined by counting 1 ml samples in 5 ml of Picofluor in a tri-carb liquid scintillation spectrometer. The values were expressed as counts/min. Quenching was monitored by external standardization.

Results and Discussion. After labelling of the kidney with ^{3}H-(−)-NA, phentolamine (6 μM) increased the outflow of tracer in response to renal nerve stimulation (3 Hz, 60 s) by 87±8% (Mean ± S.E., n = 10), and blocked the ensuing vasoconstrictor response. Raising the phentolamine concentration to 15 μM did not cause any additional increase in outflow of tracer. It has been shown that more than 80% of the radioactivity overflowing in response to nerve stimulation is accounted for by intact ^{3}H-NA in the kidney (Frame & Hedqvist 1975). It is currently believed that phentolamine increases transmitter overflow by blocking presynaptic α-adrenoceptors that mediate NA autoinhibition, while its inhibitory effect on NA uptake seems to be of little importance in this respect (cf. Langer 1979).

It has been shown that α-adrenoceptor antagonists substantially reduce or even annul the release of prostaglandin E_2 and adenosine in the rabbit kidney (Fredholm & Hedqvist 1978, Hedqvist et al. 1980). Both these substances are potent inhibitors of NA release in the rabbit kidney (Frame & Hedqvist 1975, Hedqvist & Fredholm 1976). It is not unlikely therefore that α-adrenoceptor manipulation tends to overestimate the significance of NA autoinhibition because of simultaneous interference with a feedback mechanism, operated through prostaglandin E_2 and adenosine.

In order to evaluate the relative significance of the different control mechanisms, the stimulant effect of phentolamine on NA secretion was compared with that of the prostaglandin synthesis inhibitor indomethacin, and the adenosine antagonist theophylline. Indomethacin (6 μM) and theophylline (100 μM) increased nerve-induced release of NA by 27 and 34% respectively (Fig. 1) in harmony with previous observations in this tissue (Frame & Hedqvist 1975, Hedqvist et al. 1978). It is true that theophylline is a phosphodiesterase inhibitor, but it is highly unlikely that such an effect could contribute significantly to its enhancing effect on NA release. Thus, 100 μM theophylline inhibits rabbit kidney phosphodiesterase by less than 15%

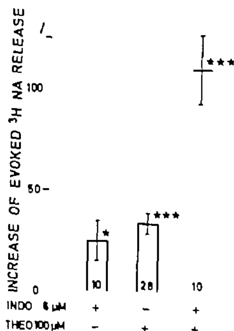


Fig 1 Effects of indomethacin (INDO) and theophylline (THEO) alone and in combination, on ³H-(−)-NA release evoked by nerve stimulation (3 Hz 60 s) in Tyrode s perfused rabbit kidneys. Mean values ± S E. number of experiments indicated in the columns. $P<0.05$ $P<0.001$

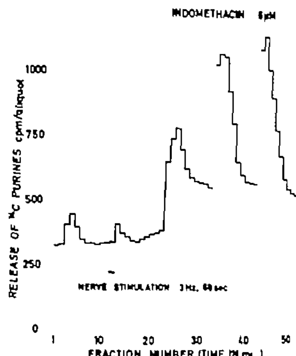


Fig 2 Tyrode s perfused rabbit kidney prelabelled with ¹⁴C-adenine Effect of indomethacin on basal and nerve induced (3 Hz 60 s) release of tracer predominantly in the form of adenosine inosine and hypoxanthine

and other more effective phosphodiesterase inhibitors do not increase NA release in the kidney (Hedqvist et al 1978) When indomethacin and theophylline were given together the nerve induced release of NA increased significantly more ($P>0.05$) than could be accounted for by the sum of their individual effects (Fig 1) A possible explanation for this synergistic effect was given in experiments in which purine release was studied

It has been shown (Fredholm & Hedqvist 1978) that infusion of labelled adenine into the renal artery results in a substantial uptake and incorporation of the label predominantly in the form of nucleotides while subsequent release both spontaneously and that induced by nerve stimulation occurs as nucleosides adenosine and its metabolites inosine and hypoxanthine After labelling of the kidney with ¹⁴C adenine indomethacin caused a marked increase in the release of tracer in all probability consisting of adenosine and its metabolites (Fig 2) Both spontaneous release and that induced by renal nerve stimulation were augmented and the effect was not specific for indomethacin but readily occurred also with two other prostaglandin synthesis inhibitors meclofenamic acid and eicosatetraynoic acid These findings suggest that removal of a prostaglandin mediated mechanism operated to restrict NA release and to attenuate vasoconstrictor responses results in increased vascular resistance in turn leading to adenosine release and inhibition of NA secretion As a consequence the use of prostaglandin synthesis inhibitors seems to represent an underestimate of removal of a prostaglandin mediated feedback mechanism operated on NA secretion in the kidney Presently it is not known whether the same holds true also for other tissues However prostaglandin synthesis inhibition is commonly associated with an increase in smooth muscle tone which is a significant stimulus for purine release (cf Hedqvist 1977 Fredholm & Hedqvist 1980)

Summing up the results indicate that NA secretion may be controlled by the transmitter itself as well as by prostaglandins and purines Since prostaglandins and purines are predominantly released from postjunctional elements they represent a transsynaptic mechanism for feedback control of NA secretion which seems particularly suitable to meet local tissue demands Moreover transsynaptic modulation seems to be at least as important as

NA autoregulation, even when disregarding that manipulation of α-adrenoceptors presumably affects both mechanisms.

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Specific sensations evoked by activity in single identified sensory units in man

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The use of lacquer insulated tungsten semimicro-electrodes for single nerve fiber recordings in awake human subjects (Vallbo & Hagbarth 1968) largely materialized the desire to compare both stimulus and sensation with the messages which pass up the sensory nerve fibres (Adrian 1931). Out of the range of scientific accomplishments innocently anticipated from the application of the microneurographic technique a few are still unmet, like for example a definitive answer to the questions—does activity in single sensory units of various types reach consciousness? and are the various sensory modalities encoded in single units? Uncertainty in this regard derives from the fact that various natural stimuli that can be applied to the skin normally activate several units with adjacent or overlapping receptive fields (Johansson & Vallbo 1979), thus confusing the experimental situation. Through the application of a technique to induce trains of impulses in single nerve fibers we have found it possible to compare sensation with the messages which pass up single sensory units in awake human subjects and thus demonstrate specific conscious percepts related to impulse activity in identified single sensory units in man.

The technique is based upon *intraneural microstimulation* and subsequent recording, through the same electrode, of the receptor characteristics and conduction velocity of individual sensory units. Our observations indicate that it is possible to activate single nerve fibers by this technique as it is possible to record impulses from single fibers. Confidence upon fulfilment of the single unit requisite is gathered from the observation of stringent criteria. Having ensured that, after nerve impalement, the electrode tip allows very selective and stable A and C single nerve fiber recording, the examiner explores minutely the nerve while delivering initially a 3 Hz train of short (0.25 ms) pulses at a learned supraliminal intensity (0.3–0.9 V). The subject, instructed to report the occurrence of sensory events referred to the territory of the pertinent nerve eventually signals some faint sensation. When such sensation is focally localized in a single and discrete skin area, the examiner upon delivery of natural stimuli to the skin is consistently able to identify in that area and usually nowhere else, the receptive field of a sensory unit whose evoked afferent impulses can be picked up from the tip of the exploring electrode now used for recording. Although natural stimulation of the receptive field must co-activate several units, usually only the protagonist unit which is in focus for the electrode can be recorded (Fig 1). Intensity of the repetitive intraneural stimulus is then minimized into a region just above perceptual threshold where a pure sensation is captured, like pressure vibration, etc with a pure temporal dimension which can be either continuous or intermittent. An all or nothing behaviour for the percept is demonstrated when the pure focal sensation does not grow in intensity nor area within a small range of stimulus intensities, thus reflecting unitary excitation. Proof that the fiber stimulated and the fiber recorded are one and the same can be obtained by physiologically marking the stimulated fiber: prolonged, high frequency intraneural microstimulation can render that single myelinated sensory fiber hyperexcitable (Torebjörk & Ochoa, unpublished) as it does with motor fibers (Bergmans 1973). Upon intraneural recording this fiber will either discharge spontaneously or will generate burst in response to a triggering pulse. Impulses evoked from the receptive field of that particular unit only will now interfere with or trigger activity in the hyperexcitable unit (Fig. 1). Thus, it can be certified that a recorded unit with defined receptor characteristics and conduction velocity is identical with the unit stimulated in the nerve. By this manoeuvre the identity has been proven for 51 mechanoreceptive units, classified as rapidly adapting (RA) Pacinian corpuscle units (PC) or slowly adapting units of various types (SA I SA II)

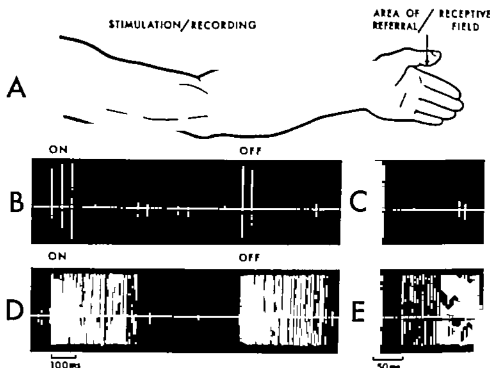


Fig 1 Microelectrode stimulation and recording from the same single sensory unit (A) Intraneural microstimulation in the median nerve at elbow is referred as flutter vibration to a focal area in the thumb (B) Intraneural recording detects on and off responses in a single RA unit to skin indentation in the area of referral (C) Electrical stimulation in the receptive field of the RA unit excites a fiber with a conduction velocity of 45 m/s (White arrow indicates stimulus artifact.) (D–E) Prolonged intraneural microstimulation at high frequency induces post-tetanic hyperexcitability which marks the stimulated fiber Mechanical (D) and electrical (E) stimuli in the receptive field of the RA unit now evoke sterotyped bursts of impulses Time bases are common for B–D and for C–E respectively

It will be realized that the intraneural microstimulation method for functional isolation of single afferent units selects for those that do arouse a conscious percept and if as long suspected there are units whose individual activity never reaches consciousness or units who only do so within a special frequency range they would pass undetected For this reason the method is complemented by the routine identification of single units by conventional means where rather than primarily microstimulating units intraneurally and selecting by percept the examiner primarily excites units by natural stimulation of receptors while recording intraneurally thus selecting by electrophysiological criteria. Having established the response characteristics of an isolated unit the electrode is used to stimulate its fiber and the percept is established while depriving the subject of audiovisual cues as to the stimulus parameters

From the combination of methods it has emerged that trains of impulses in single RA units in the glabrous skin of the hand usually reach conscio ness whereas activity in single SA II units prac cally never does Fairly often trains of impulses single SA I units can be perceived The situati for C nociceptor units is less certain because natural conglomeration of their axons in bundl within Schwann cells and their frequent associati with A delta fibers within nerve fascicles (Och 1976) Thus although excitation of C fibers and A delta fibers has usually aroused conscious sens tion it is difficult to ascertain whether the percept induced by one or several units

Together with localizing an exclusive recept field the subject of course volunteers a perceptu quality Its description is concrete to begin wi (painless–painful intermittent–continuous dul bright) and then qualified (pressure vibratio stinging pain dull pain itch) The trained sul ject soon learns to predict accurately the respons characteristics of the protagonist sensory units. Fo example painless tapping which becomes inte

[illegible] flutter or vibratory buzzing at higher stimulation frequencies, is typically underlied by activity in RA (or PC) units with fast conducting myelinated fibers, as claimed by Talbot et al (1968) in collateral experiments with monkey and man. Similarly painless continuous pressure is evoked by repetitive impulses in SA I units with fast fibers. Sharp stinging pain has so far been found served by high threshold nociceptive units with slow myelinated fibers in the A delta range, and dull pain by [illegible] nociceptive units with conduction properties typical of unmyelinated (C) afferent fibers (Torebjörk 1974). There is nothing bizarre to the quality of the sensations evoked intraneurally: they are comparable to the familiar ones elicited via receptors by adequate stimuli. But the qualities of the sensory modalities experienced after natural stimulation, and the qualities of the sensations evoked when receptors are bypassed by intraneural microstimulation are not equivalent. For instance the intermittent tactile sensation evoked from RA units is devoid of any pressure component, and the constant pressure sensation evoked by 10 Hz stimulation of SA-I units is devoid of tactile intermittency. This means that the percepts induced by intraneural microstimulation of single sensory units are pure and specific whereas sensory modalities evoked by natural stimuli to the skin, like touch may be a blend from activity in sensory units of more than one type.

A preliminary note on the sensations aroused from microstimulation in motor (muscle) nerve fascicles is pertinent. Tension and dull pain can be perceived deeply from muscle but the physiological characteristics of the corresponding afferent units remain to be established. Occasionally pain is referred not only to the appropriate muscle territory. For instance, microstimulation in a median nerve fascicle innervating the flexor muscles in the forearm caused pain perceived as arising also from the cardiac region. Such remote referral obviously implying convergence along the pain pathways has not been observed when stimulating cutaneous fascicles in the upper limb.

As predicted by Adrian & Zotterman (1926) modulation of impulse frequency in a single unit may be enough to signal intensity of a stimulus. Indeed, firing frequency is translated into *intensity* of cutaneous percepts (pressure, pain) but determines the *frequency* of intermittent percepts (flutter vibration). The sensory modality does not change with frequency. The irrelevance of frequency modulation). The sensory modality does not change with frequency. The irrelevance of frequency modulation in terms of sensory modality coupled with the specific sensations evoked by impulse activity in single units of different types as defined by their receptor characteristics, provides definitive proof for specificity in the sensory system while further questioning the Pattern Theory (Sinclair 1955).

It is anticipated that the technique of intraneural microstimulation will facilitate the identification of various types of sensory units in microneurographic recordings in man, and that it will provide a powerful tool to study the peripheral determinants for spatial temporal and intensity resolution of sensations from skin and muscle.

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Leukotriene B_4 A highly potent and stereospecific factor stimulating migration of polymorphonuclear leukocytes

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Arachidonic acid is transformed via the cyclo-oxygenase catalyzed pathway into prostaglandins (including prostacyclin) and thromboxanes (Samuelsson et al. 1978). Recently a novel group of arachidonic acid derived compounds, the leukotrienes (LT), has been discovered (Samuelsson et al 1980, Samuelsson & Hammarström 1980) Thus 5-hydroperoxy-eicosatetraenoic acid (5-HPETE) formed by lipoxygenase catalyzed oxygenation of arachidonic acid at C 5 can be transformed into the unstable epoxide intermediate leukotriene A_4 (LTA_4). Enzymatic hydrolysis of LTA_4 yields LTB whereas two 5 1 -dihydroxy isomers (compounds I and II) are formed by non-enzymatic hydrolysis of the intermediate LTC_4 which has SRS-A activity and is formed from LTA_4 by addition of glutathione can be further metabolized by elimination of the γ glutamyl residue into LTD LTC_4 and LTD have been identified in SRS-A from various sources. They are both potent bronchoconstrictors and increase the permeability of microvasculature (Dahlén et al. 1980)

We have earlier demonstrated formation of 5-HETE, LTB_4 (including the two non-enzymatically formed isomers) and LTC in human leukocytes (Samuelsson et al 1980, Hansson & Rådmark 1980 In view of the earlier reported effects of lipoxygenase products on chemotaxis in human leukocytes (Goetzl 1980) we have studied the effects of the various arachidonic acid derived products on chemotaxis and the response of human PMNLs to the chemotactic peptide fMLP in the presence of cyclo-oxygenase and lipoxygenase inhibitors

Leukocyte preparation The erythrocytes in heparinized blood (10 IU heparine, without preservative, per ml blood) were sedimented in 4 5% dextran T 500 and the supernatant withdrawn and centrifuged at 500×g for 6 min. The cellular pellet was washed twice in heparinized 0 9% saline (1 IU heparine per ml saline) and the leukocytes were suspended in a 3 1% Hepes buffer (Sigma, St. Louis, Mo., USA) dissol ed in Hank s medium to make appropriate PMNL concentrations. In some experiments PMNLs were incubated with indomethacin (Sigma) or eicosatetraynoic acid (a kind gift from Dr J Pike Upjohn Co., Kalamazoo Mich., USA) for 15 min at 37°C both dissolved in ethanol at a final concentration of .5% As controls served cells incubated with solvent only

Preparation of 5-hydroxy-eicos tetraenoic acid (5-HETE) 5 12-dihydroxy eicosatetraenoic acids (LTB and compound I and II) and LTC Human PMNLs were incubated with arachidonic acid (Nu-Chek Prep. Inc Elysian Minn. USA) and ionophore A23187 (Eli Lilly Indianapolis Ind. USA) The incubate was extracted and purified by high pressure chromatography as previously described (Borgeat & Samuelsson 1979 Rådmark et al 1980)

Random migration and stimulated locomotion were assayed with a modification of a method measuring PMNL migration under agarose (Afzelius et al 1980) Six series, each consisting of 3 wells, were punched in the agarose (containing 0.08% albumin). The diameter of one well was 3 mm and the distance between the centers of two adjacent wells was 5 mm Ten μl of PMNL suspension

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Abbreviations. fMLP=formyl-Met-Leu-Phe PMNL( ) =polymorphonuclear leukocytes, ETYA=5,8,11 14-eicosatetraynoic acid, SRS-A=slow reacting substance of anaphylaxis, 5-HETE=5-hydroxy-eicosatetraenoic acid, LT=Leukotriene

Table 1 *The ability of LTB_4 LTC_4 5 HETE and compounds I and II to induce a stimulated migration of neutrophils measured as the distance to the leading front cells in mm*

The unstimulated migration never exceeded 0 13 mm in any examination Mean ± S D values for PMNLs from 5 different subjects (each sample triplets)

Conc (M)	LTB	LTC_4	5-HETE	Compound I	Compound II	fMPL
10^{-6}	0 61±0 09*	0 17±0 0?	0 18±0 03	0 66±0.30*	0 74±0..8*	–
10^{-6}	0 81±0 05	0 14±0 04	0 13±0 01	0 43±0.07*	0 71±0.14*	
10^{-}	0.56±0 07*	0 13±0 02	0 13±0 05	0 10±0 01	0 20±0 02*	0 80±0.27**
10^{-8}	0 27±0 01	0 1 ±0 02	0 10±0 03	0.13±0 04	0 13±0.04	
10^{-9}	0 13±0 0?	0 11±0 0.	0 14±0 04	0 11±0 03	0 11±0 03	–

$P<0.01$ $P<0.05$ in comparison with measures for unstimulated cells (Wilcoxon s signed rank test).
PMNLs showed a desensitized pattern of stimulated migration (Chenoweth et al 1979)
* Mean of triplets

containing approximately 10×10^{6} cells/l was filled in the central well The chemotatic factor either fMLP (10^{-7} M) in Hank s medium 5-HETE or the leukotrienes dissolved in 10% ethanol were filled in the outer well Tissue culture medium was added to the inner well

The dishes were incubated for 0.5–3 h at 37°C in an atmosphere of 5% CO After fixation with methanol and staining with hematoxylin and fuchsin the agarose dishes were removed and the distance migrated by the front was measured by microscopy and given in mm

The orientation of lamellopodia and nuclei of 300 PMNLs migrating towards the cytotaxin and tissue medium wells was estimated by microscopy The cells were considered polarized in the gradient when the nuclei were in the rear and the anterior lamellopodia located within a 90° sector open towards the cytotaxin well (Afzelius et al 1980)

Effects of incubation of PMNLs with indomethacin or eicosatetraynoic acid (ETYA) When migration of PMNLs was stimulated with 10^{-7} M fMLP preincubation for 15 min with indomethacin (2.5×10^{-4}–2.5×10^{-5} M) caused dose dependent inhibition of migration Spontaneous migration was also slightly impaired ($P<0.05$) However when the concentration of indomethacin was decreased to 2.5×10^{-6} M a significant enhancement of fMLP stimulated migration was observed ($P<0.01$ Wilcoxon s signed rank test) In concentrations 2.5×10^{-7} M or below indomethacin showed no significant effect on stimulated or spontaneous migration when compared to controls

PMNLs pretreated with ETYA for 15 min at 37°C showed markedly impaired migration when final inhibitor concentration was 2.5×10^{-4}–2.5×10^{-5} M However at 2.5×10^{-4} M ETYA many PMNLs seemed damaged according to results of trypan blue exclusion At final concentrations 2.5×10^{-6} M or below the results were comparable to those of control cells. This occurred both when migration was spontaneous or stimulated with 10^{-7} M fMLP

Effects of 5-HETE LTC_4, LTB_4 and compounds I and II When the different compounds to be tested were added to the cytotaxin well PMNL migration was significantly stimulated by LTB ($P<0.01$ Wilcoxon s signed rank test) and compounds I and II ($P<0.05$) (Table 1) When 5 HETE or LTC_4 were utilized the migration was not significantly different from that of control cells

In order to assess whether LTB and compounds I and II stimulated directed or random migration i e chemotaxis or chemokinesis we analyzed the course of migration and the degree of orientation at 30 60 and 120 min after the start of incubation and compared this with fMLP as cytotaxin PMNLs stimulated by fMLP showed both the highest degree of orientation and progression of the cell front at 30 and 60 min The orientation decreased after 120 min to control values (reference range ($\bar{x}$±2 S D) is 13 5–30 9% Palmblad et al to be published)

PMNLs stimulated by LTB also showed directed and stimulated migration at 30 and 60 min when concentrations were 10^{-6}–10^{-8} M At 10^{-6} M LTB decreased the stimulated migration presumably by causing desensitation (Chenoweth et al 1979)

With compound I directed migration was observed at concentrations of 10^{-6}–10^{-6} M and with compound II at concentrations of 10^{-}–10^{-7} M in the system employed

The observation that eicosatetraynoic acid

(ETYA) caused dose-dependent decrease of PMNL migration induced by fMLP indicates that oxygenation of arachidonic acid is essential for the chemotactic response of human PMNLs to the peptide. High concentrations f indomethacin also inhibited the chemotactic effect of fMLP where as lower concentrations of the cyclo-oxygenase inhibitor increased the chemotactic response. These findings were compatible with a role of lipoxygenase products in chemotaxis and when some recently discovered metabolites of arachidonic acid in PMNLs (5-HETE, LTC LTB and compounds I and II) were studied LTB was found to be a potent chemotactic factor. These findings confirm and extend recent reports from other laboratories demonstrating chemotactic effects of LTB (Ford-Hutchinson et al. 1980, Goetzl 1980). The non-enzymatic isomers of LTB (compounds I and II) were considerably less active than the enzymatically formed isomer of LTB and 5-HETE and LTC_4 did not show any significant effect on the migration in the system employed. The stereospecificity in the response to the dihydroxy acids provides support for physiological role of LTB as a chemotactic agent. The present result and recent studies thus indicate that the leukotrienes play a role not only in immediate hypersensitivity reactions but also in inflammation through the increase in permeability of microvasculature by LTC and LTD and the chemotactic effect of LTB.

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The observation that eicosatetraynoic acid

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